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STUDIES ON LACTATION*

I. PRODUCTION OF MILK IN THE DOG AS INFLUENCED BY DIFFERENT KINDS OF FOOD PROTEINS

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MOST of the experiments concerning the influence of diet upon lactation have been conducted for commercial purposes on cows and goats. These animals are the result of several centuries of intensive breeding, and the capacity of the mammary gland, and probably their entire metabolism, have been so specialized that we are not able to make direct comparisons of the findings in these animals with those on other animals and humans, where the process of milk secretion has not been so highly developed.

Ssubotin (1866), working with dogs, found that the kind of nourishment had a very important influence upon the composition of milk. Voit (1869), one of the earliest workers on the influence of diet upon the composition of milk, also experimenting with dogs, found that high protein feeding favorably influenced milk secretion. Although his individual experiments were not of long duration, they seem to have been complete and well controlled. The work of these two investigators, along with that of many others in their period, seems to show that the diet is an extremely important factor in determining the secretion of milk, and that the protein constituent of the diet greatly influences its composition.

Hoobler (1917) studied the effects upon human milk production of diets containing various forms and quantities of protein. The nutritive ratios varied from 1:4 to 1:15, and the different types of protein used were meat (round steak), milk, egg, cereal, and nuts. The diets of narrow nutritive ratio gave positive nitrogen balances and increases in the milk

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nitrogen, whereas the diets of wide nutritive ratio gave negative nitrogen balances and, as Hoobler states, the effect was that of "milking the flesh off their backs." It was hard to keep the mothers in positive nitrogen balance on vegetable protein, no matter how small the nutritive ratio. On the animal protein diets, the mothers stayed in positive nitrogen balance in ratios up to 1:6. When he calculated the efficiency of the diets, he found the greatest efficiency in a diet having a ratio of 1:4, and containing two-thirds or more animal protein. This work not only brings out the fact that relatively high animal protein diets are beneficial, but that the quality of the protein is of great importance.

One of the very few adverse reports in the literature is that of Hartwell (1922b) who found that large quantities of protein fed to a nursing rat are detrimental to the young even when the diet contains all of the essential constituents. In a lactating rat the amount of protein constituting an excess varies with the type of protein and with the individuality of the rat. Simmonds (1924) critically examined Hartwell's work and believed that her evidence was not conclusive enough to allow her to state that high protein diets are injurious. Simmonds herself was not able to produce any injurious effects when the protein constituted 67 per cent of the diet. Hartwell (1924) later on, in her study of the effect of protein excess in a lactating rat's diet, came to the following conclusions: "It is impossible to state what constitutes an excess of protein in a lactating rat's diet, because there is a quantitative relation between protein and vitamin B (complex). Thus, provided the vitamin B is increased when the protein is increased, there should be no evil effects. In the lactating rat the function of vitamin B is primarily concerned with the metabolism of protein *qua* protein or with the nitrogen part of the molecule; for to a diet of 15 grams of bread and 5 grams of protein must be added far more vitamin B than to a diet of 15 grams of bread and 5 grams of starch. A high proportion of protein (15 grams of bread to 21 grams of food casein) in the lactating rat's diet produces no harmful effect in the offspring, provided the diet is very rich in vitamin B, but the rate of growth of the sucklings is impaired."

If such a relation be true, it may well account for some of the rather slight results obtained by feeding high quality proteins. The good effects of the protein may have been masked by an insufficient supply of vitamin B.

Watson (1907), observing the influence of an excess of meat diet upon lactation in rats, states that a meat diet affects prejudicially their powers of reproduction and lactation, and in the article he suggests that the increase in consumption of animal food in England may be an important

factor in the causation of the decreased birth rate, and the diminished powers of lactation. On the other hand Hitchcock (1926) has shown that female rats that were nursing litters weighed more and raised larger and healthier litters, whose growth rate was faster, when they were fed meat in addition to an adequate balanced diet.

Sherman and Muhlfeld (1922) fed breeding rats upon diets containing respectively one-sixth whole milk powder to five-sixths whole wheat, and one-third whole milk powder to two-thirds whole wheat. Young were reared on both diets, and both diets can be regarded as adequate for growth, reproduction and successful suckling of the second generation. The larger proportion of milk in the second diet resulted in an improved nutrition for the lactating mothers. Here again the fact that animal proteins are better suited to the synthesis of milk than are vegetable proteins is brought out. Sherman and Campbell (1924) also found an increase in the milk constituents of the diet to give evidence of an improved nutrition in the lactating rat mother. Cary and Meigs (1924) cite the work of Morgen and his collaborators who showed that changes in the dietary protein affected the milk yield through a wide range in the level of the protein feeding. Cary and Meigs' own work shows that milk secretion in cows is markedly affected by changes in the dietary protein.

Hart and Humphrey (1919) state that the only safe procedure for the maintenance of high milk production in cows is through the use of a high plane of protein intake which, although of low relative efficiency, should be drawn from the plant protein concentrates rather than from those of animal origin. It is only natural to suppose that an herbivorous animal should derive its necessary protein material from a plant source rather than from an animal source.

In searching for some specific protein responsible for lactation, Hartwell (1922a) states that her experiments suggest that edestin is a very good protein, and that it improves the growth curve of suckling rats when the mother's diet is deficient in protein, but, when added to a diet containing an adequate amount of protein, it has no effect.

Adair (1925) has obtained significant results in connection with human mothers. He observed 400 cases in which he studied the weight changes in the babies for twelve days after birth. The babies were weighed before and after feeding to determine the amount of milk taken from the mother. The efficiency of breast feeding was determined by the effects on the offspring. He placed a hundred mothers on each of the following diets; high protein, high carbohydrate, high fat, and balanced. The following table shows the intake ratio of one diet to another.

The high protein diet and the hospital diet showed the highest progressive increase in the amount of milk secreted. Excess feeding had no stimulating effect upon quantitative secretion. The high caloric diet caused an increase in the mothers' weight, which was associated with decreased milk secretion. He therefore concluded from this work and later observations that liberal amounts of protein in the diet are desirable for human lactation.

TABLE I

Diet	High protein	(Hospital) balanced	High carbohydrate	High fat
Calories	23	30	32	39
Protein	42	31	31	33
Fat	85	104	132	207
Carbohydrate	107	141	189	152

EXPERIMENTAL

From the foregoing account of the literature we learn that milk production is definitely altered by changes in the quality and the quantity of the protein in the diet. Fairly large amounts of animal protein increased lactation in humans, rats and dogs. The questions that now arise are, can diet definitely affect the quality and quantity of milk produced over the entire lactation period; and what is the best source of protein available for lactation?

Cary and Meigs (1924) believe that the changes in the dietary protein affect milk secretion largely by inducing changes in the quantity and the quality of the amino acid mixture circulating in the blood plasma. Therefore, if we insure a good quality of amino acids in the blood, we should be able to stimulate the mammary gland to its maximum efficiency. Milk, to insure the best nourishment for the young, must contain a complete protein. Since the immediate precursors of the milk constituents are in the blood, by feeding a complete protein we should be able to produce the same in the milk. As has been shown by Hart and Humphrey (1915), milk is one of these proteins. Because there is this direct relationship between the dietary constituents and those of the milk, it is only natural that milk in the diet would be an excellent source of protein. But this does not exactly solve the question of what proteins stimulate milk secretion.

The liver is an organ that probably has many more functions than those already discovered. Therefore it is probably more significant as a food

than many other animal proteins. It has been studied in connection with blood regeneration by Whipple and his collaborators and many others. Since milk production seems to be intimately linked with the quality of the blood, it might be expected that liver would be an excellent source of protein for milk secretion.

Kidney is also a glandular organ of the body and has been shown by Mitchell and Beadles (1926, 1927) and Hoagland and Snider (1926) to have the same biological value as liver. Kidney may also be of some importance in milk secretion.

Egg has been shown by Mitchell and Carman (1924) to have a biological value, as estimated by nitrogen balance studies with rats, of 93 as compared with 77 for both liver and kidney (1926, 1927). This, along with the fact that eggs as well as milk are products of reproductive changes, would indicate that egg protein might be influential in stimulating milk production.

In order to study the effects of these various proteins, it was thought best to use the dog as the experimental animal. Boston bull dogs were chosen because their size makes them small consumers of food, they are of a quiet and friendly disposition, they are clean because of their short hair, and they are good milk producers. For the most part pedigreed stock was used in an attempt to control as far as possible the hereditary factor which, no doubt, plays a very important part in the production of milk. It was planned to use dogs of as nearly the same size and age as possible, to breed the females for the most part to the same sire, and to keep the habits of life as regular as possible, making sure that one dog received no more exercise than another. During pregnancy the mothers were allowed the freedom of the room for about one hour a day. The remainder of the time they were confined to metal dog cages, with the exception of a few short walks in the open air. In all cases the litters were limited to three pups, two male and one female. These dogs generally have five or six pups to a litter, but it was thought that by limiting the litter to three, we would allow for any casualties at birth, and also be assured that the mother would not be over-taxed at any particular period of lactation. The experiments justified this limitation for in all cases the three pups alloted to the mother were well able to take care of all of the milk produced. The pups were chosen at birth, so that their total weights would conform as closely as possible to that of the pups of earlier experiments.

Criteria of milk production. Many methods, both direct and indirect, have been used to determine the quality and quantity of milk produced by mammals. Direct quantitative collection and chemical analysis is the

method employed with such as dairy cattle, where one has no trouble in obtaining large quantities of milk. But where one is dealing with smaller mammals, it is often very difficult to obtain enough milk for analysis, and one must resort to the rate of growth of the offspring. In humans the milk production is often judged by weighing the infant before and after each feeding, thus permitting the natural emptying of the breast and also allowing the infant directly and naturally to benefit by the milk produced.

Dogs were chosen for this study partly because it would be possible to apply both direct and indirect methods. The growth changes in the pups were studied over the period of the first four weeks of lactation, and then the pups having been separated from the mother during the fifth week, the milk was collected quantitatively by manual manipulation four times a day, care being taken that the glands were drained completely at every milking. The milk of each milking was placed in a small stoppered bottle and put in the ice box. At the end of the day these were pooled and the day's milk analyzed. The constituents varied very little from day to day over this short period. The marked daily variations that have been noted in cows were not apparent in these experiments with dogs.

Another important criterion of the efficiency of a lactation diet is the nutritive condition of the mother. Therefore metabolic studies were done during the ninth week of pregnancy, third week of lactation, fifth week of lactation and sexual rest. The blood as well as the urinary chemistry was studied in an attempt to note any physiological changes that might occur during these periods.

Methods of analysis. Carmine was fed as a marker for the fecal excretion at these periods before the morning meal. The mothers were catheterized and blood was drawn, also before the meal, to insure basal nutritive level. The mothers ate all of the food with few exceptions, sometimes requiring some coaxing or pampering on the part of the feeder. The urine was collected in 1000 cc. flasks containing a few drops of toluene (for preservative), and analyzed for total nitrogen by the Kjeldahl method; ammonia-nitrogen, Folin (1925) method; amino acid nitrogen, Henriques-Sörensen (1910) formol titration method; total sulfur, Benedict's (1909) method; total phosphorus, Fiske and Subbarow (1925).

The feces were collected in a covered jar of 95 per cent alcohol, dehydrated to dryness by heating on a steam bath after several applications of 95 per cent alcohol, pulverized, and analysed for total nitrogen by the Kjeldahl method. Blood analyses were done immediately upon collection. Approximately 7 cc. of the blood were centrifuged in a graduated centrifuge tube, thus determining the volume per cent of the corpuscles and plasma. Two cc. of the plasma were used for blood lipid determination by Bloor's (1928) method. Five cc. of the fresh blood were used in the preparation of Folin-Wu blood filtrate (Haden's modification, 1923). The following determinations were made upon the filtrate: blood sugar, Benedict (1926) method; non-protein nitrogen, micro-Kjeldahl method (Folin and Wu, 1919); and amino acid nitrogen, Folin (1922) method.

The milk was analysed for specific gravity by means of a 10 cc. specific gravity bottle; fat by the Babcock method; total proteins by the Kjeldahl method; casein and albumin by coagulation,

and the Kjeldahl method; total solids and ash by the usual methods; and lactose by Benedict's (1911) method.

Diets. The mother was started on the experimental diet approximately one week after conception, and continued on this diet through the fifth week of lactation. The diets were fed during pregnancy in order to be sure that the dog's metabolism had definitely adjusted itself to the experimental diet, before studying the effects of such a diet upon lactation, and to insure the same nutritive foreground for the development of the mammary glands, as was used in the study of their milk production. This procedure may be partially fallacious in that the results may also include the effect of the diet upon the intrauterine life of the pups. However, we feel that this procedure is better than one which does not provide for a similar nutritive foreperiod.

The diets were constructed according to Cowgill's suggestion (1923). He suggests a caloric intake of from 70 to 80 calories per kilo. per day; 0.8 gram of nitrogen per kilo.; 25 per cent by weight of fat; 0.4 gram bone ash; 0.2 gram salt mixture. This allows a nutritive ratio of from 1:3.1 to 1:3.5, thus permitting a liberal supply of protein.

The diets were as nearly identical as possible except for the source of protein. They were constructed upon a kilo. basis according to the plan shown in Table II.

TABLE II

0.7 to 0.8 gram of nitrogen per kilo	40% carbohydrate 2 to 3% variation
75 to 80 calories per kilo. (4–9–4 basis)	6% ash 2 to 3% variation
30% protein 2 to 3% variation	Nutritive ratio of 1:3.1 to 1:3.5.
25% fat 2 to 3% variation	

Tables III, IV, V, VI and VII show the details of the diets used. All quantities are in grams per kilo.

TABLE III

Diet No. 1

Food	Total	Protein	Fat	Carbohydrate	Water	Ash
Egg	9.00	3.937	3.821	—	0.810	0.3726
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	—	—	—	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato juice	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	19.77	4.168	3.976	5.937	4.584	1.0336
−water	4.584					
Solid weight	15.186					
% by weight	—	27.4	26.1	39.1	—	6.8
Calories per kilo. total of 77	—	17	36	24	—	—

Nutritive ratio 1:3.5 .666 grams nitrogen per kilo.

TABLE IV

Diet No. 2

Food	Total	Protein	Fat	Carbohydrate	Water	Ash
Liver	22.20	4.528	0.999	0.377	15.806	0.3552
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	2.70	—	2.700	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato juice	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	35.67	4.759	3.865	6.134	19.580	1.0162
−water	19.58					
Solid weight	16.09					
% by weight	—	29.5	24.0	39.2	—	6.3
Calories per kilo. total of 79	—	19	35	25	—	—

Nutritive ratio 1:3.1 .761 grams nitrogen per kilo.

TABLE V

Diet No. 2a

Food	Total	Protein	Fat	Carbohydrate	Water	Ash
Liver	19.30	3.937	0.868	0.327	13.735	0.3086
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	2.94	—	2.942	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.464	—	—	—	—	0.4640
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato juice	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	33.074	4.168	3.976	6.264	17.509	1.0336
−water	17.509					
Solid weight	15.565					
% by weight	—	26.7	25.5	40.2	—	6.6
Calories per kilo. total of 78	—	17	36	25	—	—

Nutritive ratio 1:3.6 .666 grams nitrogen per kilo.

TABLE VI

Diet No. 3

Food	Total	Protein	Fat	Carbohydrate	Water	Ash
Round steak	21.67	4.530	2.297	—	14.692	0.2167
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	1.402	—	1.402	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	33.842	4.761	3.865	5.837	18.466	0.8777
−water	18.466					
Solid weight	15.376					
% by weight	—	30.8	25.1	37.9	—	5.7
Calories per kilo.	—	19	35	24	—	—
total of 78						

Nutritive ratio 1:3.1 .761 grams nitrogen per kilo.

TABLE VII

Diet No. 4

Food	Total	Protein	Fat	Carbohydrate	Water	Ash
Kidney	27.31	4.533	1.310	0.109	20.946	0.3277
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	2.40	—	2.400	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt Mixt.	0.20	—	—	—	—	0.2000
Tomato	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	40.48	4.764	3.876	6.046	24.720	0.9887
−water	24.72					
Solid weight	15.76					
% by weight	—	30.2	24.6	38.4	—	6.3
Calories per kilo.	—	19	35	24	—	—
total of 78						

Nutritive ratio 1:3.1 .762 grams nitrogen per kilo.

In the case of the egg diet, the egg used was a pure, dried whole hen's egg.[1] One pound of dried egg is equivalent to approximately three and one-half dozen shell eggs. Due to the high fat content of the egg, this diet contained no lard, the fat being nearly entirely supplied by the egg. In preparing the egg for feeding, the necessary weighed amount of dried flakes was covered with water and allowed to stand in the ice box over night. It was then in a state similar to that of freshly beaten eggs.

The meat for the other diets was obtained fresh every two or three days. The visible fat and connective tissue were removed, and the meat ground and fed in this manner in connection with the other constituents of the diet.

The sucrose and corn starch supplied the necessary carbohydrates of the diets, while lard was used to supply the additional fat needed.

Patch's cod liver oil was used to insure a good supply of vitamins A and D, and incidentally make up the fat quota; Fleischmann's dried baker's yeast to insure the presence of vitamins B and G; tomato juice pressed from canned whole tomatoes to supply principally vitamin C; and chopped fresh green lettuce as a source of vitamin E.

A good grade of bone ash supplied roughage (to insure firm fecal matter) and helped make up the ash percentage.

The salt mixture used was that suggested by Cowgill (1923) as shown in Table VIII.

TABLE VIII

Salt	Grams	Per cent
NaCl	.1050	38.0
Mg citrate	.0905	32.5
KH_2PO_4	.0336	12.2
$CaHPO_4 \cdot 2H_2O$	.0215	7.8
KCl	.0192	7.0
Fe citrate	.0050	1.8
KI	.0013	0.5

The increasing caloric need, due to the growing fetuses, was met by increasing the entire diet 20 per cent at the beginning of the ninth week of pregnancy. From then on, to meet the demands of lactation, the diet was stepped up as follows:

At parturition.....25% increase over maintenance
Start of 2nd week of lactation.....50% increase over maintenance
Start of 3rd week of lactation.....75% increase over maintenance
Start of 4th week of lactation.....100% increase over maintenance

By studying the nitrogen balances and the mother's weight changes over various periods it seemed, if we can accept a positive nitrogen balance and a maintained weight as criteria of sufficient food requirement, that this change was sufficient to meet the increasing metabolic demands of the mother.

RESULTS

The results appear in the following order: Breeding, nitrogen balances, urinary analyses, blood analyses, milk analyses and growth changes.

1. Breeding and Number of Pups

Experiment 1, Dog 1, Diet 1 (egg). This dog was bred to male No. 1,

[1] Veritas, sold by Jaburg Bros. Inc., New York City.

Dec. 16, 1927. She delivered four pups, two males weighing 310 grams and 290 grams, and two females weighing 205 grams and 266 grams, respectively, on Feb. 15, 1928. The first three of these pups were used in the experiment.

Experiment 2, Dog 1, Diet 2 (liver). This dog was bred again to male No. 1, July 2, 1928. She delivered five pups, three males weighing 275, 260 and 290 grams, and two females weighing 275 and 290 grams, respectively, on September 4, 1928. The first male and the first female were discarded.

Experiment 3, Dog 1, Diet 1 (egg). This same dog was bred again to male No. 1, March 4, 1929. She delivered five pups, two males weighing 250 and 290 grams and three females weighing 255, 271 and 227 grams respectively, on May 3, 1929. The first male and the last female were discarded.

It was thought that if we could repeat the results of experiment 1, after dog 1 had been through experiment 2 (liver), we would be able to see if the diet was the main reason for the changed milk production, or whether the changed milk production was due solely to the betterment in lactation following a succeeding pregnancy. It is quite important to determine whether we are here dealing with changes in lactation due to a different age and parity, or to nutritional changes. We were unable to obtain much experimental support for the supposition that diet is the sole cause of changed milk production in this particular experiment, due to the development of infection in the mother and pups. Several of the pups developed convulsions. Owing to the infection we were unable to obtain any milk during the fifth week of lactation. We do not believe these pathological changes to be due to the diet.

Experiment 4, Dog 1, Diet 2a (liver). Dog 1 was bred again to male No. 1, April 2, 1930. She delivered five pups, two males weighing 210 and 304 grams and three females weighing 300, 274 and 264 grams, respectively, on May 31, 1930. The first male and the first female were discarded.

Since diet 2 (liver) contained a slightly greater amount of nitrogen per kilo. it was thought that this might account, in part, for the better results obtained in experiment 2 as compared with experiment 1, so in this second experiment on liver the diet was altered to conform more nearly to diet 1.

Experiment 5, Dog 2, Diet 3 (round). This dog was bred to male No. 3 about July 29, 1928, having been purchased in a pregnant state. She delivered seven pups, four females weighing 193, 213, 213 and 195 grams and three males weighing 206, 219 and 227 grams respectively, on Sept. 21, 1928. The first three females and the first male were discarded.

This dog was not a thoroughbred Boston bull terrier, but was probably

a mixture of French bull and Boston bull. She was bred to a large American bull terrier. For this reason we may not be justified in drawing an exact comparison between this experiment and the experiments on dog 1.

Experiment 6, Dog 9, Diet 4 (kidney). This dog was bred to male No. 4, Dec. 28, 1928. She delivered by caesarian section seven pups, one male weighing 200 grams and six females weighing 220, 235, 230, 205, 265 and 210 grams respectively, on Feb. 26, 1929. The mother was found dead the next day. The pups were given to a lactating American bull dog (dog 11) which had just weaned a litter of pups. She was fed on diet 4 until March 17, 1929 and samples of milk were taken for analysis. On March 17 she was changed to diet 3. Milk was taken for analysis on March 26. Her milk glands dried up on March 30. The analyses of the milk while under the influence of diet 3 are probably of no value since the mother was beginning to dry up.

There were four incomplete experiments conducted on diet 4 (kidney).

TABLE IX

Nitrogen balances. Experiment 1, Dog 1, Diet 1.

Period	Food	Urine	Feces	Milk	Balance	% Retention
	gm.					
9th week of preg. 2/4–2/12/28 inc. Total of 9 days. 20% inc. on 6th day.	61.98	39.23	7.30	—	+15.45	24.93
3rd week of lact. 2/29–3/6/28 inc. Total of 7 days.	77.48	36.06	9.11	Pups	+32.31	41.70
5th week of lact. 3/14–3/20/28 inc. Total of 7 days. Rejected 21.55 grams nitrogen of food	67.00	30.22	6.16	20.22	+10.40	15.52
Sexual rest. 5/11–5/17/28 inc. Total of 7 days. Rejected 12.65 grams nitrogen of food	31.62	28.29	3.06	—	+ 0.27	0.85

Note: Even though this dog did not eat for approximately two days during the fifth week of lactation and two days during the sexual rest period, she maintained a positive nitrogen balance.

In every case abnormal conditions developed at parturition causing the termination of the experiment.

2. Nitrogen Balances

The nitrogen balances were done primarily to see whether the increased food intake was meeting the metabolic demands in the various periods. This increase seemed to be sufficient because the experiments showed a positive nitrogen balance for all periods. The ingestion of the pups' excreta during the lactation period did not seem greatly to affect the nitrogen output. The balances in the third week of lactation are not "exact" because the mothers were secreting milk—a means of nitrogen elimination that was not deducted from the intake. However, in the fifth week of lactation when the output in the milk was considered, the animals were still in positive balance. There are greater percentage retentions of nitrogen on the liver diets as compared with the egg diet, thus indicating a better absorption and utilization of protein on the liver diets. This may be associated with the better milk production. Tables IX, X, XI and XII show the nitrogen balances.

TABLE X

Nitrogen balances. Experiment 2, Dog. 1, Diet 2.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 8/21–8/28/28 inc. Total of 8 days. Rejected 9.50 grams nitrogen of food	59.94	37.63	3.98	—	+18.33	30.58
3rd week of lact. 9/21–9/26/28 inc. Total of 6 days. Rejected 1.51 grams nitrogen of food	74.43	31.55	4.07	Pups	+38.81	52.14
5th week of lact. 10/4–10/10/28 inc. Total of 7 days. Rejected 20.21 grams nitrogen of food	81.06	36.20	4.21	26.96	+13.69	16.89
Sexual rest. 12/1–12/6/28 inc. Total of 6 days.	43.40	35.52	2.93	—	+ 4.95	11.40

Note: Even though this dog rejected approximately one day's food in each of the first three periods, she retained a positive nitrogen balance.

TABLE XI

Nitrogen balances. Experiment 4, Dog 1, Diet 2a.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 5/23–5/28/30 inc. Total of 6 days. Rejected 7.60 grams nitrogen of food	38.96	26.04	2.43	—	+10.49	26.93
3rd week of lact. 6/16–6/21/30 inc. Total of 6 days.	66.46	26.77	4.35	Pups	+35.34	53.17
5th week of lact. 6/30–7/5/30 inc. Total of 6 days.	75.96	30.59	4.78	28.45	+12.14	15.98

TABLE XII

Nitrogen balances. Experiment 5, Dog 2, Diet 3.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 9/18–9/21/28 inc. Total of 4 days.	31.08	11.72	0.92	—	+18.44	—
3rd week of lact. 10/8–10/13/28 inc. Total of 6 days.	67.99	26.14	3.96	Pups	+37.89	55.73
5th week of lact. 10/21–10/27/28 inc. Total of 7 days.	90.65	47.66	4.70	13.42	+24.87	27.43
Sexual rest. 12/15–12/21/28 inc. Total of 7 days.	45.32	31.68	2.39	—	+11.25	24.82

Note: Because the date of parturition could not be definitely estimated, the nitrogen studies for the pregnant period in this dog are not absolutely valid. The mother delivered before the metabolism period could be closed by the regular method; thus some feces and urine may have been omitted.

The results of this experiment probably are not to be compared with those of experiments 1, 2, 3, and 4, as has been stated, since this experi-

ment employed a different dog of different breeding. Table XII is shown to demonstrate that the adequacy of the diet is not peculiar to the individual dog for here with a dog of different breed and breeding we see a positive nitrogen balance throughout. The data indicate a high percentage retention of nitrogen and degree of utilization of round steak in this dog.

3. Urinary Analyses

In the study of the urinary chemistry we must bear in mind the fact that the intake was not the same for all four periods, though the percentage composition of the diet remained the same.

The slight increase in the nitrogen constituents of the urine, noted in some of the experiments during lactation, is probably due to the increased total nitrogen intake and the mothers ingesting the pups' excreta. The increased nitrogen intake probably is reflected also in the figures for sulfur and phosphorus. The ammonia and amino acid nitrogen figures are slightly higher during lactation. We know of no significance to attach to these results other than that they may be accounted for by the mothers ingesting the pups' excreta in the third week of lactation.

4. Blood Analyses

The blood analyses were done primarily to see whether there would be any significant changes in the blood chemistry due to the influence of the different dietary proteins during the lactation period. The blood sugar levels fall pretty well within the normal limits in all stages of the experiments. However the blood sugar in all cases shows a rise in the third week of lactation varying from 2 to 23 mgms. This change probably is not regular or large enough to be of any significance. There seem to be no significant changes in the non-protein nitrogen content of the blood. There is a slight rise during lactation in nearly every case. This slight rise is apparent also in the ammonia nitrogen during lactation as compared with pregnancy. As far as the corpuscular and plasma volumes are concerned, there seems to be an increased hydration of the blood during lactation. Experiment 3 shows that during the fifth week of lactation, this hydration is not apparent. There was an infection of the mammary gland causing an early drying up of the milk supply. This would lead us to believe that there is some relation between the hydration of the blood and milk production. There is a partial indication that blood lipids are higher in pregnancy and lactation in preparation for milk fat. The rise that is apparent in some cases may be only a reflection of the increased consumption of food fat during the later part of pregnancy and during lactation. If there is a

TABLE XIII
URINARY ANALYSES (TOTAL GRAMS FOR 7 DAYS)

Experiment	Dog	Diet	Analysis for	9th week Pregnancy	3rd week Lactation	5th week Lactation	Sexual Rest
1	1	1 (egg)	Total N	30.51	36.06	30.21	28.29
			NH_3 N	1.38	3.54	3.03	1.38
			Amino N	2.48	4.84	3.25	2.42
			Total S	6.26	9.30	7.08	6.91
			Total P	3.83	5.43	4.05	2.80
2	1	2 (liver)	Total N	32.93	36.80	36.19	41.44
			NH_3 N	2.14	2.22	2.33	0.81
			Amino N	2.45	3.13	3.22	2.38
			Total S	5.93	6.19	6.78	7.19
			Total P	4.00	6.04	5.46	4.24
3	1	1 (egg)	Total N	24.20	31.20	21.93	24.85
			NH_3 N	0.90	1.97	1.63	1.20
			Amino N	2.38	2.89	2.13	1.76
			Total S	6.40	8.16	7.46	
			Total P	2.80	3.29	2.48	2.38
4	1	2a (liver)	Total N	30.38	31.24	35.67	
			NH_3 N	1.80	2.39	2.39	
			Amino N	2.04	2.86	2.83	
			Total S	4.51	5.66	6.26	
			Total P	3.50	4.57	5.60	
5	2	3 (round)	Total N		30.49	47.66	31.68
			NH_3 N		0.58	1.16	1.02
			Amino N		0.77	1.80	0.95
			Total S		4.96	8.05	4.73
			Total P		1.47	2.43	1.75
6	9	4 (kidney)	Total N	31.17			
			NH_3 N	0.87			
			Amino N	1.36			
			Total S	5.61			
			Total P	2.73			

quantitative relationship between the quantity of blood lipids and the percentage amount of fat in the milk, it is not at all borne out in these experiments because the blood lipid values are all higher on the egg diet than on either the round or liver diets. Very slight changes in the quantity of fat in the diet have no influence upon the fat content of the milk. The diet containing the most fat and showing the highest blood lipid value produced milk with the lowest fat content.

TABLE XIV
BLOOD ANALYSES (MG. PER CENT)

Experiment	Dog	Diet	Analysis for	9th week Pregnancy	3rd week Lactation	5th week Lactation	Sexual Rest
1	1	1 (egg)	Sugar	88.89	111.11	100.00	88.90
			N.P.N.	30.00	26.08	30.77	23.10
			Amino N	7.00	5.55	8.09	8.90
			Corpuscles %	45.00	35.00	36.00	44.00
			Plasma %	55.00	65.00	64.00	56.00
			Tot. Lipids	730.57	975.50	992.40	631.00
2	1	2 (liver)	Sugar	108.00	111.00	87.00	90.00
			N.P.N.	24.00	33.30	30.00	35.70
			Amino N	7.77	11.00	11.60	11.60
			Corpuscles %	38.70	33.33	37.00	40.00
			Plasma %	61.30	66.66	63.00	60.00
			Tot. Lipids	594.59	468.74	551.52	523.64
3	1	1 (egg)	Sugar	91.00	93.00	87.00	90.00
			N.P.N.	27.27	37.50	36.36	33.30
			Amino N	9.30	11.60	13.30	8.90
			Corpuscles %	35.00	34.00	46.00	45.00
			Plasma %	65.00	66.00	54.00	55.00
			Tot. Lipids	729.00	545.00	755.00	
4	1	2a (liver)	Sugar	88.80	111.10	104.00	
			N.P.N.	22.41	25.00	24.00	
			Amino N	7.49	9.79	10.00	
			Corpuscles %	35.30	34.00	37.50	
			Plasma %	64.70	66.00	62.50	
			Tot. Lipids	663.80	646.90	775.31	
5	2	3 (round)	Sugar		96.30	93.80	93.80
			N.P.N.		24.00	27.20	38.70
			Amino N		8.75	8.40	11.60
			Corpuscles %		33.00	38.00	48.00
			Plasma %		67.00	62.00	52.00
			Tot. Lipids		405.40	461.99	444.25
6	9	4 (kidney)	Sugar	102.00			
			N.P.N.	35.20			
			Amino N	8.75			
			Corpuscles %	28.00			
			Plasma %	72.00			
			Tot. Lipids	622.00			

5. *Milk Analyses*

Probably the most striking feature is the quantitative collection. On the liver diets (2 and 2a) 1934 cc. and 2159 cc. respectively were collected over a week's period as compared with 1361 cc. collected on the egg diet and 865 cc. on the round diet.

Since we think of the percentage fat composition of the milk along with the quantity secreted, we find a higher fat percentage (13.5% and 13.25%) in the milk produced by the liver diets (2 and 2a) than that (12.25%) produced by either egg (diet 1) or round (12.5%) (diet 3). The other changes in the composition of the milk may or may not be significant. It is interest-

TABLE XV
MILK ANALYSES (GRAMS PER CENT)

Experiment	Dog	Diet	Analysis for	3rd week Lactation	5th week Lactation
1	1	1 (egg)	Sp. Gr.	1.033	1.030
			Fat	12.75	12.25
			Total solids	23.58	22.65
			Ash	1.16	1.24
			Proteins	7.74	9.46
			Casein		5.62
			Albumin		2.32
			Lactose	3.79	3.20
			Vol. in cc. for 7 days		1361.0
2	1	2 (liver)	Sp. Gr.	1.039	1.037
			Fat	13.25	13.50
			Total solids	22.90	22.50
			Ash	1.04	1.11
			Proteins	7.10	8.88
			Casein		5.42
			Albumin		2.03
			Lactose	3.10	3.14
			Vol. in cc. for 7 days		1934.0
3	1	1 (egg)	Sp. Gr.	1.029	
			Fat	11.00	
			Total solids	21.52	
			Ash	0.96	
			Proteins	6.27	
			Casein	4.08	
			Albumin	1.46	
			Lactose	2.92	

TABLE XV (*Continued*)

Experiment	Dog	Diet	Analysis for	3rd week Lactation	5th week Lactation
4	1	2a (liver)	Sp. Gr.		1.032
			Fat		13.25
			Total solids		23.50
			Ash		1.07
			Proteins		8.74
			Casein		5.20
			Albumin		1.79
			Lactose		3.21
			Vol. in cc. for 7 days		2159.0
5	2	3 (round)	Sp. Gr.	1.035	1.039
			Fat	10.75	12.50
			Total solids	29.20	25.40
			Ash	1.61	1.33
			Proteins	6.34	9.70
			Casein		7.03
			Albumin		2.07
			Lactose	3.39	2.79
			Vol. in cc. for 7 days		865.0
6	11	4 (kidney)		After feeding kidney 19 days	
			Sp. Gr.	1.039	
			Fat	10.25	
			Total solids	21.53	
			Ash	1.22	
			Proteins	7.66	
			Casein	4.92	
			Albumin	1.78	
			Lactose	3.60	

ing to note, however, that the protein content is higher in the fifth than in the third week of lactation, while the total solids are slightly lower in each case. The protein content and the ash content of "egg" milk are slightly higher than those of "liver."

In experiment 6 we see that the foster mother (dog 11), when nursing the five pups of dog 9 and being fed kidney (diet 4) produced milk showing a fat content of 10.25 per cent. This experiment, of course, is not at all comparable with the others but was done in order to gain some light on the composition of "kidney" milk. It was believed that kidney might be an excellent milk stimulant. It evidently provided plenty of milk for

this mother to raise five pups after having raised a large litter of her own. The disappointing fact was that the milk did not contain as high a fat content as was expected. Since this dog was not a Boston bull, but an American bull (much larger) it is likely that these dogs do not produce a milk with so high a fat content, and that this 10.25 per cent may be in the high range of its capacity. The work quoted from Voit (1869) on the milk analysis of a dog weighing 34 kilos. showed a fat percentage of only 7.39 per cent to 10.32 per cent. It is possible that the larger species of dogs produce milk of a lower fat content than dogs of a much smaller breed, such as Boston bull dogs.

When dog 11 was changed to round diet (diet 3) she rapidly dried up. This may have been coincidental or it may have been due to the inefficiency of the round diet.

6. Growth Changes

We note, from the plotted growth curves of the pups (Chart 1), that the liver diet again excels. The pups in experiments 2 and 4 (liver) showed much better growth than those in the other experiments, (egg, round and kidney).

Experiment 4 duplicates experiment 2 and experiment 3 duplicates experiment 1 as far as the growth curve could be studied (infection in the latter part of experiment 3). This seems to prove that the changes in lactation were solely due to dietary changes and not the age and parity of the mother.

Chart 1 also shows that round is comparable with egg as a stimulant. Kidney is probably the least effective. These findings may not be exactly true since experiments 5 and 6 were carried out on different dogs and since the mother in experiment 6 was only a foster mother and nursed five pups instead of three.

Table XVI gives a comparison of the m values. We see that the difference between the average m for the liver diets and that for the egg diets is quite significant and that the liver is 1.34 times as good as egg, or in terms of per cent one can say that egg is only 74.57 per cent as good as liver for milk production, as judged by the growth of the pups.

If we regard the experiments done on different dogs (experiments 5 and 6) and if we are at all justified in comparing them with the first four experiments, we see that "round" ranks with "egg" and "kidney" ranks much lower. The m value for "kidney" undoubtedly would have been different had there been three instead of five pups.

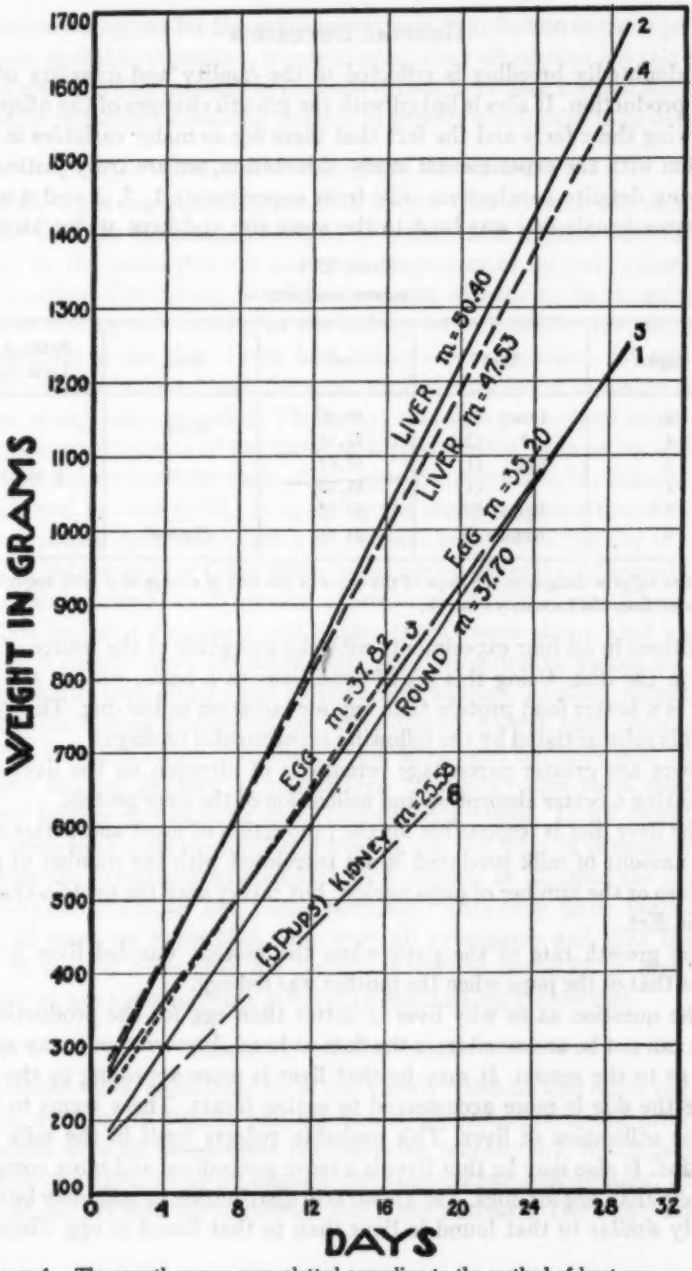

CHART 1.—The growth curves were plotted according to the method of least squares using the formula $y=mx+b$ where:

$$m=\frac{n\Sigma xy-\Sigma x\Sigma y}{n\Sigma x^2-(\Sigma x)^2};\ b=1/n(\Sigma y-m\Sigma x);\ x=\text{days old};$$

y=weight in grams; n=number of observations.

General Discussion

Undoubtedly breeding is reflected in the quality and quantity of the milk production. It also is linked with the growth changes of the offspring. Knowing these facts and the fact that there are so many variables in connection with the experimental study of lactation, we are truly justified in drawing definite conclusions only from experiments 1, 2, 3 and 4 where the same female dog was bred to the same sire and kept under identical

Table XVI
Growth Analysis

Expt.	Diet	m*	Aver.	Ratio of m Liver:Egg
2	Liver (2)	50.40	48.96	1.341:1
4	" (2a)	47.53		
3	Egg (1)	37.82	36.51	
1	" (1)	35.20		
5	Round (3)	37.70		
6	Kidney (4)	25.58	(5 pups)	

* The value m designates the slope of the curve or the rate of change of y with respect to x, calculated from the formula $y=mx+b$.

conditions in all four experiments with the exception of the source of protein in the diet. Using this set of conditions as a basis, we can say that liver is a better food protein than egg for lactation in the dog. This statement is substantiated by the following experimental findings.

There are greater percentage retentions of nitrogen on the liver diets indicating a better absorption and utilization of the liver protein.

The liver diet is responsible for the production of more and better milk. The amount of milk produced is not correlated with the number of pups in utero or the number of pups nursed, but rather with the protein changes in the diet.

The growth rate of the pups when the mother was fed liver is 1.34 times that of the pups when the mother was fed egg.

The question as to why liver is better than egg for the production of milk can not be answered from the data at hand. However, one may speculate as to the reason. It may be that liver is more appealing to the dog, since the dog is more accustomed to eating meats. There seems to be a better utilization of liver. This probably reflects itself in the milk production. It also may be that liver is a more generalized and more complete protein than egg for dogs. The amino acid distribution in milk may be more nearly similar to that found in liver than to that found in egg. Since the

quantitative figures for the entire amino acid distribution in these proteins are not available at present, we are unable to say whether or not this factor is responsible. Still another hypothesis is that liver may contain some hormone or lactation-promoting vitamin in greater abundance than does egg.

Special Remarks on the Kidney Diet

Of all of the experiments started on kidney (diet 4) none was successful (other than the freak experiment 6). Whether these failures were brought about by the toxicity of the kidney in pregnancy or by mere coincidence is a question. The kidney diet did not seem at all toxic to the lactating dog 11. Concerning the toxicity of the kidney let us consider the manner of preparation of the diet. Fresh beef kidneys were obtained, fat and connective tissue removed, and the meat washed with water to insure the removal of any adherent urine. The kidney was then ground and mixed with the other constituents of the diet just before feeding. It is rather unlikely that the kidney had any toxic effect upon the organism. McCollum, Simmonds and Parsons (1921) in studying the nutritive properties of certain animal tissues state that there is no distinct evidence of toxicity in either muscle, kidney or liver tissue when fed at planes of intake sufficiently high to introduce from 35 to 70 per cent of protein in the diet of rats. These same workers and also Hoagland and Snider (1926) have shown that kidney, liver and muscle have approximately identical nutritive values for rats. These results may or may not be applicable to dogs. Another supposition is that the kidney stimulated the intra-uterine growth thus causing the pups to be much larger than they would have been normally and so preventing natural delivery at parturition. In many cases there were deformed fetuses and in some cases hydrocephalic pups. In all except one case there were quite enlarged mammae in the last part of pregnancy, indicating the promise of an abundant milk supply. In all cases the mother was unable to deliver her young normally. This may have been coincidental since we know that they were all primiparas and that the per cent of Boston bulls that are unable to deliver normally is high. Assuredly we are not altogether justified in condemning kidney diet.

Summary and Conclusions

Balanced diets, containing all of the known vitamins and other requirements, were fed to Boston bull females of the same breed and breeding. The diets were all based upon the following scheme: 0.7, to 0.8 gram of nitrogen per kilo., 75 to 80 calories per kilo., 30 per cent protein, 25 per cent fat, 40 per cent carbohydrate and 6 per cent ash. The only variable

in the diets was the source of protein. Beef liver, round, kidney and dried hen's egg were the sources of protein.

The experimental diet was fed from the time of conception through the fifth week of lactation, the diet being increased to meet the demands of the growing pups.

The litters were all limited to three pups and the mothers given identical care.

The milk was collected by manual manipulation during the fifth week of lactation when the pups were not with the mother.

One dog was bred to the same sire and kept under identical conditions for four pregnancy-lactation periods. Egg was the source of protein in the first and third periods while liver was used in the second and fourth.

She showed a better nitrogen retention on liver both times. When fed liver she produced in the fifth week of lactation 1934 cc. of milk, containing 13.5 per cent fat in one experiment and 2156 cc. of milk, containing 13.25 per cent fat in the other. When fed egg she produced in the corresponding week, only 1361 cc. of milk containing 12.25 per cent fat. The pups of the liver experiments grew 1.34 times as fast as those of the egg experiments.

Another dog of a different breed and breeding, when fed round steak as the source of protein, produced in the fifth week of lactation 865 cc of milk containing 12.5 per cent fat. The growth of the pups was practically the same as on the egg experiment.

Owing to some unknown cause, no experiments were completed on kidney diet. This diet did not seem toxic and produced plenty of milk when fed to a lactating dog with five pups. It may have some effect on the intrauterine life of the pups, since none of the mothers fed on this diet during pregnancy delivered normally.

The chemical studies may be of some significance in the study of the physiology of lactation.

The work shows the degree of betterment in lactation that can be brought about by changing the source of protein in the diet. Liver is a much better source of protein for lactation than either egg or round steak.

Our conclusion that liver is the best source of protein (of those tried) for milk production in dogs, is borne out by the quantity of milk produced, the fat content of the milk, the nitrogen retention and the slope of the growth curve of the pups.

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THE VALUE OF THE OYSTER IN NUTRITIONAL ANEMIA

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SINCE the anouncement of Hart and co-workers (1) in 1928 that copper is effective in supplementing iron in the cure of anemia produced by feeding milk to the rat, considerable interest has been manifested in the possible supplementary rôle of various other inorganic elements. Although all other investigators (2 to 9, 43) in this field, except Drabkin and Waggoner (10) who question the specificity of copper, agree that copper supplements iron in the regeneration of blood in nutritional anemia, several workers (5, 7b) offer evidence tending to show that other elements besides copper are just as effective in their supplementary action and further (6, 7a) that a group of inorganic elements rather than any single element is concerned in blood regeneration. In these investigations, the rôle of manganese has received particular attention.

As a result of these studies, much confusion now exists in the literature as to what elements are concerned in hemoglobin regeneration in nutritional anemia. Thus, Waddell and co-workers (8) report that Cu is "unique" in supplementing Fe and that Zn, Cr, Ge, Ni, Co, Pb, Sb, Sn, Cd, Hg, As, and Mn, when fed either singly or as a mixture of these twelve elements, are ineffective. Lewis, Weichselbaum and McGhee (4) have found Mn and Co to be ineffective while Krauss (9) has recently confirmed Waddell in respect to Mn. Likewise, Underhill and co-workers (43) have recently demonstrated the ineffectiveness of Co, Ni, Zn, and Mn. On the other hand, Myers and Beard (5a, b) offer results showing that besides Cu, Mn as well as Ni, Ge, Co, As, Ti, Zn, Rb, V, Cr, Se, and Hg are effective. Goerner (2) found Mn to be potent, while Zn and Al are reported as inert. Again, Titus, Cave and Hughes (7a, b) have reported that Mn is just as effective as Cu, whereas, in accord with the results of Mitchell and Miller (6), they found a combination of Mn and Cu to be more potent than Cu alone in supplementing Fe.

This interest in the inorganic elements, particularly in Fe, Cu, and Mn,

led several laboratories to carry out analyses for these three elements on a large number of plant and animal foods. The Wisconsin workers (11 to 16) have been very active in this direction and have published results showing the Fe, Cu, and Mn content of a wide variety of both plant and animal foods. Richards (17) likewise gives values for the Mn content of various foods. In this laboratory, Remington and Shiver (18) determined the amounts of Fe, Cu, and Mn in a large number of samples of eighteen of the more commonly used vegetables grown in South Carolina. At the same time, the laboratory became interested in making similar determinations on various animal foods. In this connection attention was drawn to the oyster, which we found to contain rather large amounts of both Fe and Cu. The latter element was present in much greater concentration than was found in any plant or animal food thus far examined here. Further analysis revealed the presence of manganese also. It occurred to us, therefore, that since the oyster is such a good source of both Fe and Cu (elements admitted to be concerned in hemoglobin regeneration in nutritional anemia), it ought to exhibit marked potency as an hematopoietic agent.

A review of the literature revealed that the oyster, besides being a good source of vitamins A, B, C and D (19 to 23), contains in rather high concentration various inorganic elements, of which all except iodine have been studied in nutritional anemia. The amounts of iron (13), copper (15, 24 to 29), zinc (27, 29 to 32, 35), lead (34), arsenic (27, 33) and iodine (36, 37) contained in the oyster have been determined by several investigators. Besides these, other metallic elements are probably present.

The presence of these several elements again suggested that the oyster might be a potent anti-anemic food, and hence the present study was undertaken. Since in addition to Fe, Cu, and Mn, other elements—Zn, Pb and As—are present, we decided to compare the effectiveness of the oyster with a solution containing equivalent amounts of Fe, Cu, and Mn alone. By this procedure we reasoned that if elements other than Fe, Cu, and Mn play a rôle in blood regeneration, then the oyster would be much more effective than the solution of the three inorganic elements. In addition, an acid solution of oyster ash was fed for comparative purposes. It was expected that the results of this experimental plan would throw further light on the existing controversy as to the elements concerned in blood regeneration in nutritional anemia.

Experimental

The rats used in the experimental work were bred in the laboratory em-

ploying Sherman Diet 13 as modified by Russell (38). The composition of the breeding ration follows:

	Per Cent
Whole wheat (ground)	59.8
Whole milk powder*	29.9
Swift's meat scrap (55% protein)	9.1
Sodium chloride	1.2
	100.0

* Klim.

This ration was found to contain 125 mg. of Fe and 4.1 mg. of Cu per kg. Distilled water was given *ad libitum* to all rats. Shortly after birth, the number of rats in each litter was reduced to seven. At 21 days of age, the young rats in our colony averaged 36 grams in weight. The animals were allowed to remain with the mother until they reached an average weight of 55 to 60 grams, which they usually attained at 25 to 30 days of age. They were then separated from their mothers, grouped by litters in galvanized iron wire cages and fed exclusively on milk in order to render them anemic. At the same time, rats taken from these same litters were maintained on the stock ration throughout both the preliminary and experimental periods as controls.

Following the usual technic (39) used by other investigators, blood samples were taken from the tip of the tail. The blood, after being drawn into the blood pipette, was expelled immediately into a small test tube containing 5 cc. of one per cent HCl solution and then shaken. When all the blood samples had been taken in this manner, the test tubes were heated at 55 to 60°C for seven minutes and cooled to room temperature. This procedure, used by Mitchell and Miller (6), insured maximum color development of the acid hematin. Hemoglobin determinations were made by the Newcomer method, using a calibrated yellow glass disc in the colorimeter as a standard. Following the experience of Waddell and co-workers (39), we dispensed with the taking of red blood cell counts in our investigation.

A total of seventy-three rats was used in the experimental work. In order to establish the average hemoglobin value of our animals at the start of the preliminary milk feeding period, five litters of rats comprising thirty-four animals were used. The average hemoglobin value for these rats was found to be 11.79 gm. per 100 cc. of blood. This value lies between the average value of 10.83 obtained by the Wisconsin workers (39) for rats 3 to 4 weeks of age weighing 50 to 60 g. and the value of 12.62 obtained by Williamson and Ets (40) for animals 20 to 39 days old.

In rendering the rats anemic, whole milk powder[1] reconstituted to liquid

[1] Klim.

milk was employed. Reconstitution was effected by adding the required amount of warm distilled water to the milk powder contained in a Mason jar. The jar was then closed securely and vigorously shaken by hand for about ten minutes.

The whole milk powder was purchased in fifty pound tins, each lot being analyzed[2] for iron and copper before using it for feeding purposes. In all, five lots of milk powder were used throughout the experimental work. The iron and copper content of the different lots of dry milk varied slightly. Expressed in terms of liquid milk, the values varied from 1.0 to 1.4 mg. per liter for Fe, and from 0.20 to 0.27 mg. per liter for Cu. Assuming an average daily intake of 35 cc. of milk per rat, this amount of milk would afford the animal 0.035 to 0.050 mg. of Fe and 0.007 to 0.009 mg. of Cu. The distilled water used in reconstituting the milk introduced only negligible amounts of Fe and Cu. Drabkin and Waggoner (10) also used powdered whole milk[1] to produce anemia in their rats.

The time required to produce a marked anemia on milk varied from 6 to 8 weeks. At the end of this time, the average hemoglobin value for all the milk-fed animals had decreased to 5.68 gm. per 100 cc. of blood, the individual values ranging from 3.19 to 7.80 gm. per 100 cc. Simultaneously, seven litter-mate control animals that had been kept on the stock ration for the same length of time yielded an average hemoglobin value of 15.3 gm. Hemoglobin determinations were also carried out on a group of twenty-eight stock rats representing four litters from our breeding colony. These animals (84 to 89 days old) were approximately the same age as the anemic rats at the end of the preliminary milk feeding period. The hemoglobin values for this group on the stock ration averaged 14.84 gm. per 100 cc. of blood with a range in values of 14.03 to 16.81 gm. per 100 cc. Williamson and Ets (40) reported a hemoglobin value of 13.54 for normal rats 80 to 99 days of age. The blood of our anemic rats at the start of the curative period contained only 38 per cent of the normal content of hemoglobin.

At the start of the curative period, the rats were placed in individual galvanized iron wire cages. There were altogether 66 anemic animals, of which number 16 rats, representing various litters, were retained on milk *only* throughout the curative period to serve as negative controls. The remaining 50 anemic rats were divided into groups and fed the various test materials to be described, while the seven animals fed the stock ration during the preliminary period were maintained on this same diet throughout the experimental period to serve as normal controls.

[2] All analyses for the iron, copper or manganese content of materials employed were carried out by the methods used by the Wisconsin workers (12 to 14, 41).

The oysters used in the present investigation were obtained from various beds located along the coast of South Carolina. Shucked oysters from these localities were brought to the laboratory, dried in porcelain evaporating dishes first over a steam bath and finally in an electric oven. Table I below shows the iron, copper and manganese values for six samples of oysters. A

TABLE I

SHOWING THE IRON, COPPER AND MANGANESE CONTENT (DRY BASIS) OF OYSTERS GROWN ALONG THE COAST OF SOUTH CAROLINA

Sample No.	Source	Iron (p.p.m.)	Copper (p.p.m.)	Manganese (p.p.m.)
423	Charleston County (Bull's Bay)	760	93	34
648	Charleston County (Bull's Bay)	633	52	15
649	Beaufort County (Daufuski Island)	929	67	26
652	Beaufort County (Daufuski Island)	1035	40	58
653	Beaufort County (Bluffton)	740	34	46
731	Georgetown County (Winyah Bay)	510	28	29
	Range in values	510–1035	28–93	15–58
	Average value	768	52	35

study of the values in this table reveals that there is much variation among the samples. Hiltner and Wichman (27) in 1919 analyzed oysters from eight different beds situated at or near the localities indicated in Table I. Using the iodometric method for the determination of copper, these investigators reported values ranging from 23 to 52 p.p.m. (fresh basis). For comparison with the results of our copper analyses, these values would be, expressed on a dry basis, approximately 161 to 364 p.p.m. When the same samples were examined for zinc, Hiltner and Wichman found amounts ranging from 182 to 502 p.p.m. (fresh basis).

For the feeding experiments, a composite sample (Sample 1) representing a mixture of three dried oyster samples was made so as to have sufficient material to last throughout the entire experimental period. On analysis, the mixed sample yielded the following values: Fe, 892 p.p.m.; Cu, 58 p.p.m. and Mn, 20 p.p.m. In choosing the various levels of dried oysters for daily feeding to the rats, 0.56 gm. was selected as the highest dosage since this amount furnished 0.5 mg. of Fe and 0.0327 mg. of Cu—amounts which have been shown by different workers to be fully adequate for blood regeneration in nutritional anemia. Another dried oyster sample (Sample 2) was also used in the feeding tests, this sample containing 760 p.p.m. of

Fe, 93 p.p.m. of Cu and 34 p.p.m. of Mn. Table II shows the various levels at which the two samples were fed, together with the daily amounts of Fe, Cu and Mn furnished by each dosage.

TABLE II

SHOWING THE AMOUNTS OF FE, CU, AND MN (DRY BASIS) CONTAINED IN THE DAILY OYSTER SUPPLEMENTS

Sample No.	Amount of dried oysters	Equivalent on a fresh basis	Iron	Copper	Manganese
	gram	gram	mg.	mg.	mg.
1	0.56	4.0	0.500	0.0327	0.0112
1	0.28	2.0	0.250	0.0164	0.0056
1	0.14	1.0	0.125	0.0082	0.0028
2	0.16	1.7	0.125	0.0149	0.0054

For comparative purposes in the feeding tests, three concentrations of an HCl solution of the oyster ash of Sample 1 were prepared in such a manner that 1 cc. of each solution corresponded respectively in Fe, Cu, and Mn content to the three levels of dried oysters (Sample 1). Since besides Fe, Cu, and Mn other workers (27, 29 to 35) have found rather large amounts of Zn, Pb and As in the oyster—elements concerning the rôle of which Waddell and co-workers (8) and Myers and Beard (5a, b) offer contradictory evidence—a comparison of dried oysters (Sample 1) and of the oyster ash solution was made with a prepared solution[3] containing only Fe, Cu, and Mn, in order to determine whether or not elements other than Fe, Cu, and Mn are concerned in hemoglobin formation.

The mineral supplements were fed daily to the rats in the morning, at which time no milk was given until the supplement was entirely consumed. In the case of the 1 cc. liquid supplements, these adjuvants were mixed with about 3 to 5 cc. of milk before feeding. The rats consumed the supplements readily after which they were given milk *ad libitum* until the next morning. The experimental feeding period lasted eight weeks.

The results obtained in the feeding experiments are summarized in Ta-

[3] This solution was prepared in the following manner. Using C. P. iron wire, a $FeCl_3$ solution was made from which the Cu was precipitated by H_2S. $CuSO_4$ and $MnCl_2$ solutions were used to represent Cu and Mn. On analysis, these three solutions were found to contain negligible amounts of Fe, Cu, and Mn as contaminants. From these sources, solutions were prepared corresponding in Fe, Cu, and Mn content to the various levels of dried oyster and oyster ash solution. The required amount of the three elements was contained in 1 cc. of each solution.

ble III and are shown graphically in Chart 1.[4] From a study of Table III, it is to be observed that the average hemoglobin content of the blood of the sixteen negative control rats on milk alone decreased from 6.4 gm. per 100 cc. at the start of the experimental period to 4.5 gm. per 100 cc. at the end of this period at which time only nine rats survived. The average values for the group of seven normal control animals representing rats maintained on the stock ration throughout both the preliminary and experimental periods remained practically the same.

The data in the table also clearly indicate the marked effectiveness of the oyster in hemoglobin regeneration. The three levels of dried oyster Sample 1 (0.56 gm., 0.28 gm. and 0.14 gm.) all exhibited the capacity to

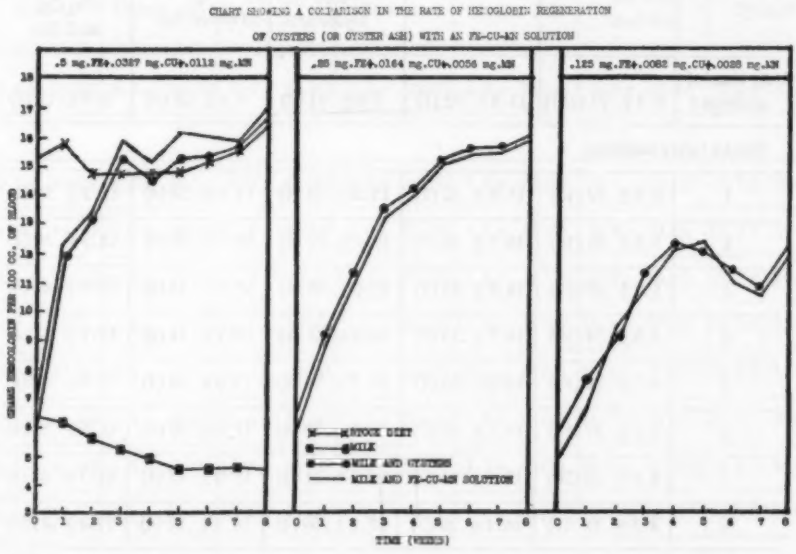
CHART SHOWING A COMPARISON IN THE RATE OF HEMOGLOBIN REGENERATION
OF OYSTERS (OR OYSTER ASH) WITH AN Fe-Cu-Mn SOLUTION
.5 mg.Fe+.0367 mg.Cu+.0112 mg.Mn
.25 mg.Fe+.0164 mg.Cu+.0056 mg.Mn
.125 mg.Fe+.0082 mg.Cu+.0028 mg.Mn
GRAMS HEMOGLOBIN PER 100 CC. OF BLOOD
STOCK DIET
MILK
MILK AND OYSTERS
MILK AND Fe-Cu-Mn SOLUTION
TIME (WEEKS)

raise the hemoglobin content of the anemic rats' blood. Accepting for comparative purposes 14.8 gm. hemoglobin per 100 cc. as the average value for our normal animals, it is evident from a study of the values in the table that daily dosages of 0.56 gm. and 0.28 gm. of dried oysters permitted complete blood regeneration in 2 to 3 weeks and 4 to 5 weeks, respectively. On the other hand, a level of 0.14 gm., although it permitted a considerable degree of blood regeneration, nevertheless failed to bring the hemoglobin

[4] Since both dried oysters and oyster ash gave practically the same response in hemoglobin regeneration, their values are plotted as one group in the chart.

TABLE III

AVERAGE WEEKLY HEMOGLOBIN VALUES* AT TIME SUPPLEMENT WAS ADDED AND AT INTERVALS OF DRIED OYSTERS, AN ACID SOLUTION OF OYSTER ASH AND A SOLUTION CONTAINING ONLY

	Negative Controls on Milk	Normal Controls on Stock Diet	Sample 1		
			0.500 mg. Fe 0.0327 mg. Cu 0.0112 mg. Mn		
			Furnished by		
			.56 gm. Dried Oysters	HCl Soln. of Oyster Ash	Solution Containing Fe, Cu, and Mn
At time of addition†	6.4±.21 (16)‡	15.3±.32 (7)	5.0±.41 (4)	5.3±.28 (4)	6.0±.16 (4)
Weeks (after addition)					
1	6.2±.22 (16)	15.8±.32 (7)	11.8±.32 (4)	11.8±.58 (4)	12.2±.52 (4)
2	5.5±.20 (16)	14.7±.14 (7)	13.7±.06 (4)	12.7±.38 (4)	13.3±.38 (4)
3	5.2±.28 (15)	14.8±.22 (7)	15.0±.48 (4)	15.5±.14 (4)	15.9±.43 (4)
4	4.8±.24 (13)	14.7±.14 (7)	14.0±.21 (4)	15.1±.14 (4)	15.1±.35 (4)
5	4.5±.29 (12)	14.8±.34 (7)	15.5±.71 (4)	15.0±.24 (4)	16.3±.43 (4)
6	4.5±.27 (11)	15.2±.29 (7)	15.0±.50 (4)	15.4±.30 (4)	16.1±.38 (4)
7	4.6±.28 (10)	15.5±.11 (7)	16.2±.21 (4)	15.0±.48 (4)	15.7±.49 (4)
8	4.5±.28 (9)	16.4±.30 (7)	17.2±.69 (4)	16.3±.57 (4)	17.1±.51 (4)

* Expressed as grams of hemoglobin per 100 cc. of blood.

† After 6 to 8 weeks of preliminary feeding on reconstituted milk only.

‡ The figures in parenthesis refer to the number of rats used in obtaining the average hemoglobin value.

level up to the normal value. At the end of the eight weeks period, the average hemoglobin content of the blood of the rats fed this dosage reached only 12.0 gm. per 100 cc., representing approximately 80 per cent regeneration. A similar partial recovery was obtained with 0.16 gm. oyster Sample 2 which in comparison with 0.14 gm. of oyster Sample 1 furnished the same amount of Fe but about twice as much of Cu and Mn. At the conclusion

TABLE III (*continued*)

VALS THEREAFTER, OF ANIMALS RECEIVING VARIOUS AMOUNTS OF FE, CU, AND MN IN THE THE THREE ELEMENTS

Sample 1						Sample 2
0.250 mg. Fe 0.0164 mg. Cu 0.0056 mg. Mn			0.125 mg. Fe 0.0082 mg. Cu 0.0028 mg. Mn			.125 mg. Fe .0149 mg. Cu .0054 mg. Mn
Furnished by			Furnished by			Furnished by
.28 gm. Dried Oysters	HCl Soln. of Oyster Ash	Solution Containing Fe, Cu, and Mn	.14 gm. Dried Oysters	HCl Soln. of Oyster Ash	Solution Containing Fe, Cu, and Mn	.16 gm. Dried Oysters
5.6±.23 (6)	6.3±.16 (6)	5.8±.20 (6)	6.3±.20 (5)	4.9±.24 (6)	4.8±.23 (6)	5.4±.27 (3)
9.7±.49 (6)	8.5±.28 (6)	8.9±.41 (6)	8.2±.38 (5)	7.4±.28 (6)	6.7±.25 (6)	8.5±.13 (3)
11.2±.49 (6)	10.9±.38 (6)	11.2±.57 (6)	8.9±.38 (5)	9.7±.30 (6)	9.4±.34 (6)	10.3±.35 (3)
13.5±.44 (6)	13.5±.18 (6)	13.2±.24 (6)	11.3±.30 (5)	11.4±.33 (6)	10.9±.26 (6)	11.1±.24 (3)
13.7±.18 (6)	14.4±.27 (6)	14.1±.35 (6)	12.6±.44 (5)	12.6±.38 (6)	12.1±.37 (6)	12.7±.26 (3)
15.0±.37 (6)	15.3±.33 (6)	15.2±.39 (6)	12.1±.48 (5)	12.1±.48 (6)	12.4±.57 (6)	11.7±.11 (3)
15.7±.48 (6)	15.5±.68 (6)	15.4±.55 (6)	11.5±.50 (5)	11.4±.39 (6)	11.0±.66 (6)	11.3±.35 (3)
15.7±.42 (6)	15.5±.18 (6)	15.5±.25 (6)	10.8±.50 (5)	10.7±.47 (6)	10.5±.65 (5)§	10.0±.47 (3)
16.5±.22 (6)	15.4±.07 (6)	16.0±.41 (6)	12.0±.70 (5)	12.5±.84 (5)§	12.1±.62 (5)	10.8±.32 (3)

§ One rat in this group died.

of the eight weeks' supplementary feeding period, 0.125 mg. of Fe (as $FeCl_3$ solution) was added to 0.16 gm. oyster Sample 2 thereby increasing the daily intake of Fe to 0.250 mg. This Fe augmentation permitted hemoglobin regeneration to the normal level in three weeks. This finding indicates that a daily dosage of 0.125 mg. of Fe as furnished by 0.16 gm. of dried oyster Sample 2 is insufficient for complete blood regeneration in the rat, despite the rather large amount of Cu—0.0149 mg.—which accompanied it. Further proof of this finding is indicated by the fact that 0.28

gm. of oyster Sample 1 which furnished approximately the same amount of Cu and Mn, but twice as much Fe as 0.16 gm. of oyster Sample 2, cured anemia in 4 to 5 weeks.

From the above results, it is evident that the minimum effective level of dried oyster Sample 1 lies between 0.14 gm. and 0.28 gm. or, on an undried basis, between 1.0 gm. and 2.0 gm. Since the amount of Fe furnished daily by the milk was approximately 0.035 to 0.050 mg. the results indicate that the minimum daily Fe requirement of the rat lies between 0.17 mg. and 0.30 mg. when supplemented with the amounts of Cu and Mn used in these experiments.

A further study of the data given in Table III shows that the dried oysters (Sample 1), the acid solution of oyster ash (Sample 1) and the prepared solution containing *only* Fe, Cu and Mn all brought about hemoglobin regeneration at approximately the same rate[5] when compared at the three different levels. This result indicates, first, that the inorganic elements present in the oyster are responsible for its hemoglobin regenerating capacity and second, that the antianemic potency of the oyster can be accounted for on the basis of its content of the three elements, Fe, Cu, and Mn.

Discussion

In the light of the controversy concerning the indispensability of elements other than Fe and Cu in blood formation, the above experimental results offer some pertinent evidence. Mitchell and Miller (6), in a study comparing a spinach extract fed at a level containing 0.5 mg. Fe, 0.014 mg. Cu and 0.012 mg. Mn with two prepared solutions containing in the one, 0.5 mg. Fe and 0.05 mg. Cu, and in the other, 0.5 mg. Fe, 0.05 mg. Cu and 0.05 mg. Mn, found the extract to be more potent in blood regeneration than the Fe-Cu or the Fe-Cu-Mn complex of pure salts. They also reported that the Fe-Cu-Mn complex was more effective than the Fe-Cu combination, indicating that Mn plays a rôle in hematopoiesis. When these same investigators subjected the spinach extract to qualitative analysis and found the following elements present—Cu, Sb, Sn, Fe, Al, Zn, Mn, Sr. Ca, Mg, Na, K and P—they concluded that rather than Cu alone there is a group of elements that is active in supplementing Fe in hemoglobin building. Myers and Beard (5a, b) also offer evidence indicating that several elements besides Cu and Mn are concerned in blood formation. On the other hand, the results of our investigation would appear to be more in

[5] Calculations of the significance ratios revealed that the differences between the mean values are not significant.

line with those of Waddell and co-workers (8) who in tests on several elements reported that Cu is "unique" in supplementing Fe. This contention appears evident since in the present experiments the prepared solution containing only Fe, Cu, and Mn allowed blood regeneration at approximately the same rate as the dried oysters and the oyster ash solution, despite the presence of Pb, Zn, and As in large amounts in the oyster. For this reason, the results obtained in this investigation would seem to warrant the conclusion that elements in the oyster other than Fe, Cu, and Mn are not concerned in blood formation.

The question whether or not Mn is necessary and aids Cu in supplementing Fe cannot be answered from the results obtained in the present experiments, but will be considered in another investigation now under way. Krauss (9) Underhill and co-workers (43) and Waddell and co-workers (8) give evidence showing that Mn is ineffective in supplementing Fe, whereas Myers and Beard (5a) and Titus, Cave and Hughes (7a) are of the opinion that Mn plays a rôle in the cure of nutritional anemia.

Sherman (42) estimates the Fe requirement of man as 15 mg. per day. On this basis, 120 g. (4.2 oz.) of oysters (undried) would furnish this daily requirement.

Summary

The finding of large amounts of Cu in the oyster prompted a study of the influence of the oyster in hemoglobin regeneration in rats rendered anemic on milk.

When fed daily for a period of eight weeks, 0.56 gm. dried oysters (4.0 gm. fresh basis) allowed regeneration to the normal hemoglobin level in 2 to 3 weeks, whereas 0.28 gm. (2.0 gm. fresh basis) brought a return to normal in 4 to 5 weeks. Fourteen hundredths of a gram (1.0 gm. fresh basis) permitted only about 80 per cent blood regeneration at the end of eight weeks. It is estimated that the minimum daily Fe requirement of the rat lies between 0.17 mg. and 0.30 mg.

A comparison of 1.–dried oysters, 2.–an acid solution of oyster ash and 3.–a solution containing Fe, Cu, and Mn, was made at various levels. All three supplements permitted blood regeneration at approximately the same rate, indicating, first, that inorganic elements present in the oyster are responsible for its antianemic potency and, second, that the effectiveness of the oyster in nutritional anemia can be accounted for on the basis of its Fe, Cu, and Mn content.

The question whether or not Mn is necessary and aids Cu in supplementing Fe cannot be answered from the results of these experiments.

Addendum

After this paper had been submitted to the editors, an abstract by Whipple and Wolf appeared (Amer. Jour. Physiol., Proc. Amer. Physiol. Soc., 1931, xcvii, 569) in which oysters are reported to have marked curative effects in milk anemia in rats. No results were reported as to the mineral composition of the oysters used.

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THE UTILIZATION OF THE IRON OF PROTEIN FOODS BY THE ALBINO RAT

(A) A COMPARISON OF THE GROWTH AND THE IRON ASSIMILATION AS AFFECTED BY DIFFERENT PROTEIN FOODS: (B) A COMPARISON OF PROTEIN FOODS SUPPLEMENTARY TO MILK AS SOURCES OF IRON IN NUTRITION*

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INTRODUCTION

THIS paper reports results of a continuation of a study by R. C. Miller, E. B. Forbes, and C. V. Smythe, which was discussed in an early number of this journal (27), the present investigation serving to extend the scope of the inquiry, and to review the earlier studies, by repetition, with the advantage of more critical procedures than were employed in the earlier work.

The general subject of the inquiry, namely "the development of evidence as to the specific values of meats, in comparison with other protein foods, as sources of iron in nutrition" was the same as in the earlier study, as also was the distinctive feature of basing conclusions as to the values of the diets as sources of iron upon determinations of the total iron content of the bodies of the experimental subjects. In the earlier study these iron estimations, however, were related only to the fresh weight, while in the studies here discussed they were related also to the dry weight and to the fat-and-water-free weight.

In the earlier study the protein foods compared were milk, muscle, kidney, brain, liver, beans, eggs, and peanuts; and in the present study this list was extended by the inclusion of pecans and English walnuts.

As in the earlier study these foods were used, in one series of tests, as sole sources of protein and iron, and a new series of tests was conducted in which the protein foods of interest were used not as sole sources of protein and iron but as supplements to milk, for these purposes.

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In the earlier as well as in the present work the rats were protected from vitamin deficiencies by the administration of appropriate vitamin-containing supplements; from energy deficiency by the employment of lactose, starch, and hydrogenated vegetable fat[1]; and from calcium deficiency by the inclusion of calcium carbonate in the rations which contained no milk, —thus determining that the protein foods would be compared only as sources of protein and iron, without confusion with other factors.

Important studies have been published on comparisons of the protein values of foods; but none, so far as the writers are aware, except their own earlier work, from the two-fold point of view of the present study.

The complication of the problem by the combination of the protein and the iron factors renders it difficult to draw conclusions, but this illogical procedure may be justified as necessarily incident to the consideration of valuable, associated attributes of natural foodstuffs.

The comparisons of protein values of foods, to which allusion has been made, will be briefly reviewed as contributions to the general background of information relating to the subject of interest.

Osborne and Mendel (1, 2) demonstrated that beef skeletal muscle and pig's heart, kidney and liver were suitable and adequate as sources of protein for nutrition, when other essential ingredients were present in the diet in sufficient amounts.

McCollum, Simmonds and Parsons (3, 4, 5) also studied the proteins of meats, and concluded that the proteins of beef kidney, liver and muscle, when serving as sole sources of protein, and when properly supplemented with all necessary factors other than protein, possessed about the same biological value as the proteins of the wheat kernel,—kidney, however, having a higher biological value than the other meats. When fed at high planes of intake these tissues gave no evidence of toxicity. The proteins of each were of about equal value as supplements to the proteins of cereals, and were superior to the proteins of milk in supplementing the proteins of certain seeds and of the potato. The deficiency of meats in calcium was recognized.

Mitchell and associates (6, 7, 8, 9, 10), using a modification of the Thomas method, determined the so-called biological value of the nitrogen of certain protein foods, including meats. The nitrogen of beef heart, kidney and liver was found to be utilizable to about the same extent, the biological values being 74 for heart, and 77 for kidney and liver. A value of 74 was determined for pork muscle, and 69 for beef muscle. Later work (11) indicated that a high content of connective tissue in beef made it less valuable as a source of protein. The biological values determined for meat foods were lower than those determined for milk, i.e. 83 to 86, and for eggs, i.e. 93, but higher than that for navy beans, i.e. 38.

Mitchell and Beadles (12), using the paired feeding method, found that the proteins of lean beef were not deficient in cystine, for the growth of rats, but concluded that the proteins of navy beans and milk were deficient in this amino acid.

Hoagland and Snider (13) studied the relative values, for maintenance and growth, of the protein of voluntary muscle, heart muscle, liver and kidney, from cattle, sheep and swine, using a method based on that of Osborne, Mendel and Ferry (14), in which is determined the relation between the nitrogen intake and the growth of young rats for periods of 30 and 60 days.

Pork liver fed for 30 days at a 10 per cent level of intake gave a somewhat higher value than

[1] Crisco.

did the other tissues, and beef liver a somewhat lower value, but these differences were less marked in periods of 60 days.

Pork heart and kidney, fed for periods of either 30 or 60 days at a 12.5 per cent level, gave higher values than did the other tissues. There were no outstanding differences among any of the tissues when all were fed at a 15 per cent level.

The authors concluded that in diets containing adequate quantities of protein, these tissues have approximately the same value for maintenance and growth.

Slonaker and Card (15, 16) made a study on rats restricted to a diet derived largely from plant sources, compared with others on a similar diet supplemented with meat protein. In general the rats which received meat excelled those on the restricted diet, in growth, reproduction, and duration of life.

Hitchcock (17, 18, 19) reported that the addition of meat to a well balanced diet already containing adequate protein increased the rate of growth of rats, and the efficiency of their utilization of food, and also improved the performance of rats in reproduction.

MacLeod (20) published preliminary experiments showing that an increase from 10 grams to 40 grams of fresh meat per rat per week improved reproduction and lactation. Dried meat was less favorable in its effect on reproduction than was fresh meat, and an increase in the proportion of dried meat in the ration from 3 per cent to 12 per cent seemed to favor reproduction and lactation, though the evidence was slight in extent. The increase in the milk in the basal diet from 12 per cent to 16 per cent allowed successful reproduction on both 3 per cent and 12 per cent of meat, the greater number of young being reared on the diet with 12 per cent meat.

Clayton (21, 22) found it difficult to evaluate for reproduction and lactation, the proteins of kidney, liver, milk and eggs, because of apparent vitamin deficiencies.

Nelson and associates (23, 24, 25) secured excellent growth and reproduction, but poor lactation, with rats consuming a meat ration supposedly fortified with vitamins, containing lean beef muscle as the main protein constituent.

Robscheit-Robbins (26) has reviewed the literature relating to meats, iron and other factors as affecting hemoglobin regeneration, and though the present evidence on this subject is not entirely clear and harmonious the relatively high value of animal tissues, liver and kidney in particular, for hemoglobin building, is obvious; and iron receives continued consideration as of value in the nourishment of the blood.

The general effect of these observations is to represent meats as efficient and non-toxic sources of protein in nutrition; with high supplementing value for the protein of certain vegetable foods; with favorable influences in growth, blood building, reproduction and lactation; with biologic values lower than those of milk and eggs; but with superiority to milk and to beans as sources of cystin.

Preliminary work at this Institute (27) showed in part (a) that the iron content of the bodies of albino rats was apparently normal when they had been fed diets in which meat foods constituted the sole source of protein, (b) that low iron content of the rats' bodies resulted from the feeding of diets in which the protein was supplied by hen's eggs, peanuts, beans or whole cow's milk, and (c) that all of the protein foods which were studied seemed to be superior to milk as sources of dietary iron.

It was possible, however, to question the apparent specific effects of the protein foods studied, since the gains in body weight, with the several

treatments, were very different, and since the nature of these gains, for instance, as to fat and water contents, was not determined. The investigation here discussed was conducted to clear up this uncertainty and to extend the evidence on all aspects of the problem.

The present report deals with two series of feeding experiments with rats, Series 1 serving to compare beef muscle, beef liver, beef kidney, peanuts, eggs, pecans and English walnuts, as supplementary sources of iron, when added in quantities supplying equal amounts of protein, to diets in which approximately the entire protein requirement was already supplied by skimmed milk powder. Conclusions were based on increase in body weight, and on determinations of the dry matter, the fat-free dry matter, and the iron of the bodies of the rats.

Series 2 was a continuation of the previous study (27), and compares meat and other protein foods as the sole sources of iron and protein. The general plan and scope of the experiments is outlined in Table I.

Experimental Procedure

The methods of preparation of the protein foods, except for the method of grinding, were the same as those previously employed (27). In both series the fresh meats were ground in a phosphor-bronze food chopper, which was nearly iron free, and dried in a Freas oven at about 85° C. In Series 1 the dried meats and the other protein foods were further ground in the above mentioned phosphor-bronze food chopper, but those used in Series 2 were ground in a porcelain mortar. The beans were cooked in water until tender. The water was then evaporated off, and the extract dried with the beans. The eggs were beaten and then dried. The peanuts were roasted, and the pecans and English walnuts were fed in the fresh state.

The experimental subjects were albino rats. They were fed individually and caged in such manner that they did not have access to their excreta.

The plan of feeding was the same as that previously used (27) except that equal quantities of feed were given daily, throughout an experimental week. The vitamin supplements were fed separately, Harris's yeast vitamin being administered as pellets, in the feed cup, and cod liver oil was given by mouth with a medicine dropper.

The rats were weighed at weekly intervals, and the quantity of feed to be given was computed on the basis of these weekly weights, seven daily portions being weighed at one time, and stored in tin boxes.

The quantity to be fed was determined by the expected consumption of the lot which received the least palatable feed, and the quantity of feed

TABLE I

PLAN OF EXPERIMENTATION

Series No.	Diet No.	No. of rats	Duration of experiment	Dietary sources of protein	Protein content of diets	
					Per cent	
1	23	5	15 weeks	Skimmed milk powder	15	
	24	5	"	" " " and dried beef muscle	27	In these diets the protein required for growth was supplied by milk powder. The other foods supplied iron and additional protein.
	25	5	"	" " " " " " liver	"	
	26	4	"	" " " " peanuts	"	
	27	5	"	" " " " dried eggs	"	
	28	5	"	" " " " dried beef kidney	"	
	30	4	"	Skimmed milk powder	15	
	31	4	"	" " " and pecans	21	
	32	4	"	" " " " English walnuts	21	
2	33	5	15 weeks	Skimmed milk powder	16	In these diets the protein foods served as sole source of protein and of iron.
	34	5*	"	Dried beef brain	"	
	35	4	"	" " muscle	"	
	36	5	"	" " kidney	"	
	37	5	"	" " liver	"	
	38	5	"	" cooked beans	"	
	39	5	"	" eggs		
	40	4	"	Peanuts		

* Two members of this group died in the course of the experiment.

given to this lot, in relation to its live weight, constituted the basis for the allowance of feed to each other lot. The quantities of food given, on this basis, are presented for Series 1 in Table II, and for Series 2 in Table V. Experience gained with this method of feeding in the experiments of Series 1 made possible the more successful regulation of the food intake in Series 2. Such variations as occurred were caused mainly by the inability of the experimenters to foretell the exact quantity of food that would be consumed. A decrease in the rate of feed allowance in the experiments of Series 2 lessened this difficulty.

When this method of feed control is used the apparent palatability of the diet, which serves to determine the rate of feeding, may be fundamental, in the sense of being truly characteristic, or may be a secondary result of nutritive deficiency and consequent low state of nutrition. In this investigation the quantity of food consumed in proportion to the body weight became smaller each week until the maximum body weight was attained—as naturally prevails during growth.

Also, by this method of feeding, animals which do not grow well are restricted in food intake in comparison with animals whose growth is good, in consequence of the fact that although the quantities of food offered are in the same proportion to the body weights, in both cases, the maintenance requirement of energy producing nutriment is relatively smaller for the larger animal, thus giving it a larger quantity of food available for body increase than the smaller animal receives, in relation to body weight. This situation tends to exaggerate differences in nutritive values of foods— the greater the differences, the greater the exaggeration of these differences.

The experimental feeding periods were fifteen weeks in length, after which the rats were killed by means of chloroform. Their bodies were prepared for analysis by the removal and discarding of the alimentary tracts, and thereafter by drying for 24 hours, at 100° C., in a Freas oven. The bodies were then crushed with a pestle, and further dried over sulfuric acid in vacuum desiccators for periods up to 120 days in length. By this time the rats' bodies reached almost constant weights, but in no case did they seem to become absolutely dry. The drying was discontinued when the losses in weight had become practically negligible. The bodies were then broken up, transferred to paper capsules, and extracted with ether for 72 hours in large Soxhlet extractors. The fat obtained from this extraction was weighed; the dry, fat-free bodies were ashed in a muffle furnace; and iron was determined in the residues.

Iron was determined by the modified thiocyanate-acetone method previously used (27), with the additional modification suggested by Elvehjem

TABLE II

SERIES 1. AVERAGE NUMBER OF GRAMS OF FOOD EATEN PER WEEK, PER GRAM OF BODY WEIGHT

Diet No.	Source of protein	Week No.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
23	Skimmed milk powder	.74	.53	.51	.50	.52	.42	.39	.37	.31	.32	.29	.29	.27	.25	.25
24	Skimmed milk powder and beef muscle	.71	.67	.61	.51	.48	.44	.40	.39	.36	.30	.28	.29	.25	.25	.25
25	Skimmed milk powder and beef liver	.55	.75	.60	.48	.52	.50	.47	.47	.37	.31	.30	.29	.27	.25	.25
26	Skimmed milk powder and peanuts	.67	.56	.52	.48	.44	.40	.31	.31	.24	.31	.29	.27	.28	.24	.25
27	Skimmed milk powder and dried eggs	.93	.58	.51	.51	.49	.43	.39	.37	.32	.31	.31	.29	.27	.25	.25
28	Skimmed milk powder and beef kidney	.70	.60	.51	.51	.49	.42	.39	.37	.32	.31	.30	.29	.27	.20	.25
30	Skimmed milk powder (high fat)	.62	.42	.40	.45	.46	.37	.35	.35	.31	.28	.28	.27	.26	.21	.23
31	Skimmed milk powder and pecans	.55	.65	.48	.48	.43	.33	.31	.31	.27	.24	.23	.22	.21	.21	.19
32	Skimmed milk powder and English walnuts	.64	.63	.50	.46	.43	.35	.34	.28	.26	.26	.25	.21	.23	.21	.18

(28), whereby any pyrophosphate present is converted to orthophosphate, by neutralizing the hydrochloric acid solution of the ash with dilute sodium hydroxide, and boiling for about an hour. Slightly higher results were obtained in some cases when this procedure was used, and it was therefore followed throughout these experiments. Extraction of the colored ferric thiocyanate with amyl alcohol yielded results consistently slightly lower than those obtained with the procedure employing the acetone.

Experimental Series 1

The composition of the diets fed in Series 1 is given in Table III. The proportion of skimmed milk powder in each diet was such as to supply approximately the amount of protein required for maintenance and for growth, the other protein foods being included in proportions furnishing

TABLE III

SERIES 1. COMPOSITION OF DIETS. (PARTS PER 100)*

Diet No.	23	24	25	26	27	28	30	31	32
Skimmed milk powder	44.37	44.37	44.37	44.37	44.37	44.37	44.37	44.37	44.37
Dried beef muscle	—	20.00	—	—	—	—	—	—	—
Dried beef liver	—	—	22.00	—	—	—	—	—	—
Peanuts	—	—	—	53.07	—	—	—	—	—
Dried eggs	—	—	—	—	33.70	—	—	—	—
Dried beef kidney	—	—	—	—	—	20.90	—	—	—
Pecans	—	—	—	—	—	—	—	50.00	—
English walnuts	—	—	—	—	—	—	—	—	39.40
Lactose	10.00	10.00	10.00	2.06	10.00	10.00	—	—	—
Starch	31.95	21.03	15.45	—	10.52	23.08	19.28	5.13	6.20
Crisco	13.18	4.10	7.68	—	.91	1.15	35.85	—	9.53
NaCl	.50	.50	.50	.50	.50	.50	.50	.50	.50
Iron content (per cent)	.0010	.0020	.0051	.0017	.0037	.0049	.0010	.0020	.0027

* Cod liver oil and Harris Yeast Vitamin were fed daily with each diet.

equal quantities of supplementary protein. The energy contents of the diets were equalized by means of lactose, starch and hydrogenated vegetable fat[3]. Lactose was used in place of the sucrose previously employed with the idea that it might be less readily fermentable in the intestine, but neither sucrose nor lactose seemed to be any more or less desirable than starch alone, and the latter was used by itself, in Series 2, as a source of carbohydrate.

[3] Crisco.

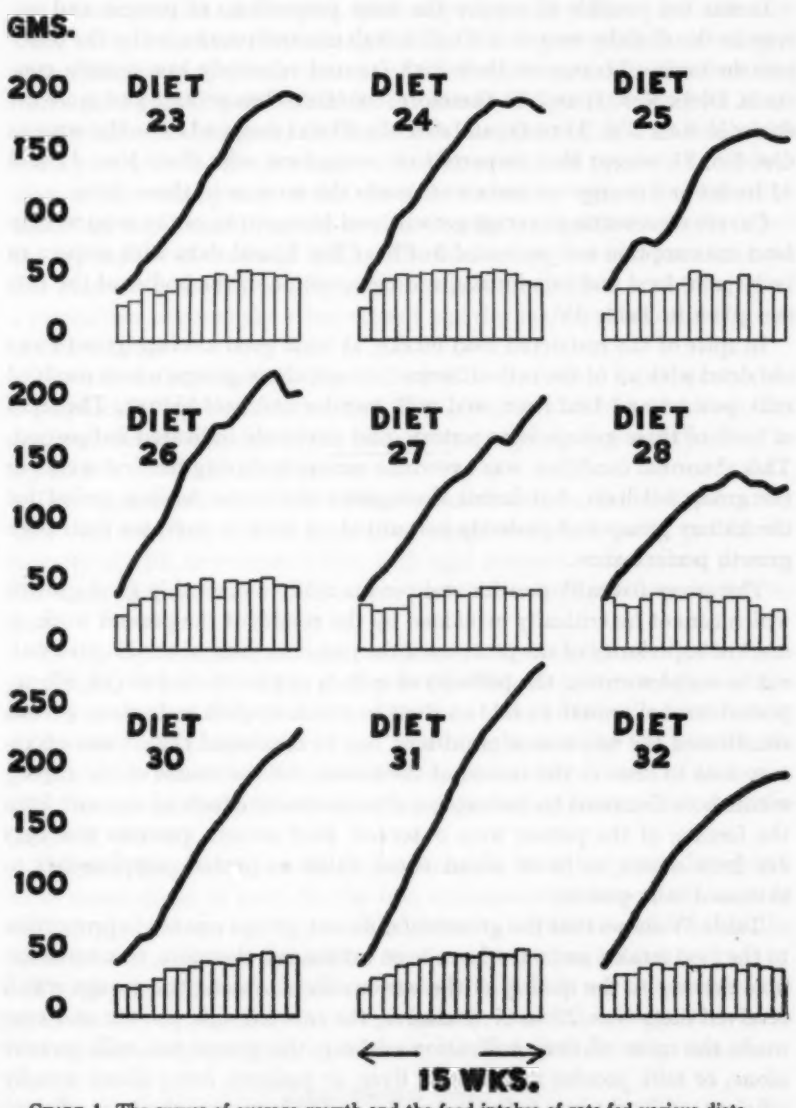

CHART 1. The curves of average growth and the food intakes of rats fed various diets.

Diet No. 23, milk; 24, milk and beef muscle; 25, milk and beef liver; 26, milk and peanuts; 27, milk and eggs; 28, milk and beef kidney; 30, milk; 31, milk and pecans; 32, milk and English walnuts.

It was not possible to supply the same proportions of protein and energy in the diets by means of English walnuts and pecans, as by the other protein foods—because of their high fat and relatively low protein contents. Diets Nos. 31 and 32, therefore, contained less protein and more fat than did diets Nos. 23 to 28; and diet No. 30 was designed to be the same as diet No. 23, except that to permit of comparison with diets Nos. 31 and 32 its fat and energy contents were made the same as in these diets.

Curves representing average growth, and histographs of the total weekly food consumption are presented in Chart No. 1; and data with respect to body gain, food and iron intake, and composition of the bodies of the rats are given in Table IV.

In spite of the restricted food intake, at least good average growth was obtained with all of the rats of Series 1 except those groups which received milk powder and beef liver, and milk powder and beef kidney. The feces of both of these groups were watery, and obviously indicated indigestion. This abnormal condition was overcome naturally during the first weeks in the group fed liver, but lasted throughout the entire feeding period for the kidney group and probably accounted, at least in part, for their poor growth performance.

The group fed milk powder and pecans made remarkably good growth which cannot be critically explained by the results of the present work. A marked superiority of the proteins of the pecan to those of the English walnut in supplementing the proteins of milk is apparent. Cajori (29, 30) reported normal growth in rats on diets in which English walnuts or pecans constituted the sole source of protein, but he concluded that it was necessary first to remove the tannin of the pecans. In the course of the experiments here discussed no indications of unfavorable effects of any sort from the feeding of the pecans were observed. Beef muscle, peanuts and eggs are here shown to be of about equal value as protein supplements to skimmed milk powder.

Table IV shows that the growth of different groups was not in proportion to the food intake, and must have been influenced, therefore, to a considerable extent, by the quality of the various diets. Among the groups which received diets Nos. 23 to 27, inclusive, the rats fed milk powder and eggs made the most efficient utilization of food; the groups fed milk powder alone, or milk powder and muscle, liver, or peanuts, being about equally efficient, while the rats fed milk powder and kidney were the least efficient in their utilization of food for growth. It should be borne in mind, in relation to these observations, that the food consumption for each experimental lot was determined, in large measure, by its growth performance.

The composition of the rats, with respect to dry matter and ether extract, reveals the fact that among the rats which grew well, there is not a wide variation in the body content of these substances. It is also true that where good growth occurred the body content of dry matter and fat is relatively high. In the case of the rats which received diet No. 28 and which made poor growth, the dry matter and fat content of the rats were lower than in any other group of the series. In harmony with the observation which has been made by many workers in relation to other animals, when the dry matter of the body was low the fat content was also low, since fat and water tend strongly to vary reciprocally; thus the data for Series 1 give a correlation between the ether extract and dry matter content of the rats of $0.91 \pm .015$.

The iron content of the bodies of the rats in the several experimental lots is graphically represented, in Chart No. 2, on the absolute, the fresh, the dry, and the fat-free dry bases, with accompanying representation of the dry matter and of the ether extract.

The representation on the fat-free dry basis most clearly and significantly shows the superiority of the meats, fed supplementary to milk, and the inferiority of milk, as sources of iron; with eggs, peanuts, walnuts and pecans, all fed supplementary to milk, occupying intermediate positions in this regard.

On the dry basis the iron content of the rats which received kidney was very high, as a result of the poor growth which these rats made, and the low fat content of their bodies; and on the absolute basis the iron content of the rats which received pecans was high because of the remarkable growth made from this diet. It is thus apparent that it was by no means only the iron content of the diets which determined the iron content of the bodies of the rats.

The percentage variations in the iron content of the body may be regarded as determined, in part, by the iron enrichment or impoverishment of the living substance, and in part as arithmetical effects of variations in the fat and water contents—which are to a large extent reciprocal.

The physiological significance of these variations is not revealed by the method of these experiments, which merely establishes suggestive facts that can be interpreted only by intensive physiological investigation.

Experimental Series 2

The composition of the diets fed in Series 2 is given in Table V. In this series the protein foods constituted the sole sources of protein and iron in the diets, and were included in such amounts as supply equal quantities

TABLE IV

SERIES 1. FOODS CONSUMED, GAINS IN BODY WEIGHT, AND COMPOSITION OF BODIES OF RATS

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. weekly food intake per gram of live weight	Av. daily iron intake	Dry matter content of body minus alimentary tract	Ether extract content of body minus alimentary tract	Iron content of body minus alimentary tract			
									Total	Per cent of fresh wt.	Per cent of dry, fat free wt.	Per cent of dry wt.
			grams	grams	grams	mgs.	per cent	per cent	mgs.	per cent	per cent	per cent
516	23	Skimmed	1.68	7.17	0.43	0.07	36.74	11.87	6.60	.0032	.013	.009
517	23	milk	1.63	7.83	0.46	0.08	41.35	17.25	4.93	.0024	.010	.006
518	23	powder	.94	5.76	0.41	0.06	38.20	12.74	4.50	.0034	.014	.009
519	23		1.12	5.71	0.40	0.06	42.42	18.07	5.02	.0034	.014	.008
520	23		1.94	8.12	0.41	0.08	39.53	15.21	6.13	.0026	.011	.007
		Average	1.46	6.92	0.42	0.07	39.65	15.03	5.44	.0030	.012	.008
521	24	Skimmed	1.00	6.21	0.42	0.12	44.44	19.92	7.41	.0054	.022	.012
522	24	milk	2.00	9.15	0.40	0.18	38.95	12.82	9.65	.0041	.016	.011
523	24	powder	1.33	7.70	0.44	0.15	39.67	13.42	7.62	.0044	.017	.011
524	24	and beef	1.59	7.81	0.41	0.16	43.29	18.95	8.91	.0052	.019	.010
525	24	muscle	1.02	5.90	0.41	0.12	43.18	18.84	7.07	.0053	.022	.012
		Average	1.39	7.35	0.42	0.15	41.91	16.79	8.13	.0049	.019	.011

526	25	Skimmed	1.87	7.50	0.42	0.38	37.59	11.39	8.86	.0043	.016	.011
527	25	milk	1.02	5.82	0.43	0.30	39.74	14.32	6.58	.0051	.020	.013
528	25	powder	1.01	6.30	0.44	0.32	38.92	12.54	7.03	.0054	.021	.014
529	25	and beef	1.06	5.89	0.43	0.30	37.94	11.43	7.36	.0056	.021	.015
530	25	liver	1.23	6.03	0.41	0.31	39.84	14.61	7.19	.0047	.019	.012
		Average	1.24	6.31	0.43	0.32	38.81	12.86	7.40	.0050	.019	.013
546	28	Skimmed	.87	5.88	0.40	0.29	31.68	3.39	6.58	.0052	.018	.016
547	28	milk	.94	6.01	0.39	0.29	31.78	3.47	6.94	.0052	.019	.017
548	28	powder	.94	5.64	0.39	0.28	34.51	7.80	5.99	.0045	.017	.013
549	28	and beef kidney	.77	4.70	0.40	0.24	31.69	4.27	5.22	.0049	.018	.015
		Average	.88	5.56	0.40	0.28	32.42	4.73	6.18	.0050	.018	.015
531	26	Skimmed	1.10	6.11	0.39	0.10	43.26	19.42	6.22	.0042	.018	.010
532	26	milk	1.17	6.04	0.36	0.10	42.23	18.15	5.58	.0035	.015	.008
533	26	powder	1.80	7.90	0.38	0.13	39.80	15.14	7.84	.0035	.014	.009
534	26	and	1.67	7.32	0.38	0.12	40.31	15.38	5.60	.0028	.011	.007
535	26	peanuts	1.83	7.92	0.37	0.14	39.41	15.08	6.46	.0029	.012	.007
		Average	1.51	7.06	0.38	0.12	41.00	16.63	6.34	.0034	.014	.008
541	27	Skimmed	1.88	6.76	0.42	0.25	37.65	12.23	7.65	.0035	.014	.009
542	27	milk	1.21	5.82	0.42	0.21	43.35	19.33	3.59	.0023	.010	.005
543	27	powder	1.19	5.51	0.41	0.20	37.06	11.14	7.72	.0051	.020	.014
544	27	and	1.30	5.83	0.41	0.22	34.81	9.89	6.33	.0038	.014	.011
545	27	eggs	2.15	7.23	0.41	0.27	37.68	12.83	8.59	.0035	.014	.008
		Average	1.55	6.23	0.41	0.23	38.11	13.08	6.78	.0036	.014	.009

TABLE IV
SERIES 1. (*continued*)

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. weekly food intake per gram of live weight	Av. daily iron intake	Dry matter content of body minus alimentary tract	Ether extract content of body minus alimentary tract	Iron content of body minus alimentary tract			
									Total	Per cent of fresh wt.	Per cent of dry, fat free wt.	Per cent of dry wt.
			grams	grams	grams	mgs.	per cent	per cent	mgs.	per cent	per cent	per cent
565	30	Skimmed	1.91	5.63	0.35	0.06	38.64	15.63	7.49	.0033	.014	.009
566	30	milk	1.50	5.73	0.34	0.06	40.02	16.78	3.61	.0020	.008	.005
567	30	powder	1.40	5.32	0.35	0.05	42.41	19.58	6.04	.0034	.015	.008
568	30	(high fat)	2.38	6.70	0.35	0.07	41.46	19.71	5.94	.0021	.010	.005
		Average	1.80	5.85	0.35	0.06	40.63	17.93	5.77	.0027	.012	.007
560	31	Skimmed	2.70	7.19	0.34	0.14	40.16	16.64	8.79	.0029	.013	.007
561	31	milk	1.62	5.17	0.31	0.10	41.97	18.74	6.12	.0032	.014	.008
562	31	powder	2.53	6.71	0.34	0.13	41.82	18.29	8.18	.0029	.012	.007
563	31	and pecans	2.57	7.26	0.34	0.15	38.98	14.56	7.88	.0028	.011	.007
		Average	2.36	6.58	0.33	0.13	40.73	17.06	7.74	.0030	.013	.007
570	32	Skimmed	1.48	5.39	0.33	0.15	37.66	13.16	5.33	.0029	.012	.008
571	32	milk	1.60	5.71	0.33	0.15	38.94	14.63	6.59	.0034	.014	.009
572	32	powder	1.44	5.65	0.36	0.15	40.89	17.38	4.82	.0027	.011	.007
573	32	and English walnuts	1.45	5.81	0.37	0.16	39.24	14.72	5.99	.0033	.014	.009
		Average	1.49	5.64	0.35	0.15	39.18	14.97	5.68	.0031	.013	.008

of protein. The energy contents of the diets were also equalized by varying the proportions of starch and hydrogenated vegetable fat. Diet No. 40 necessarily had a higher fat content and a higher energy value than did the other diets of this series, on account of the relatively high fat and low protein contents of peanuts.

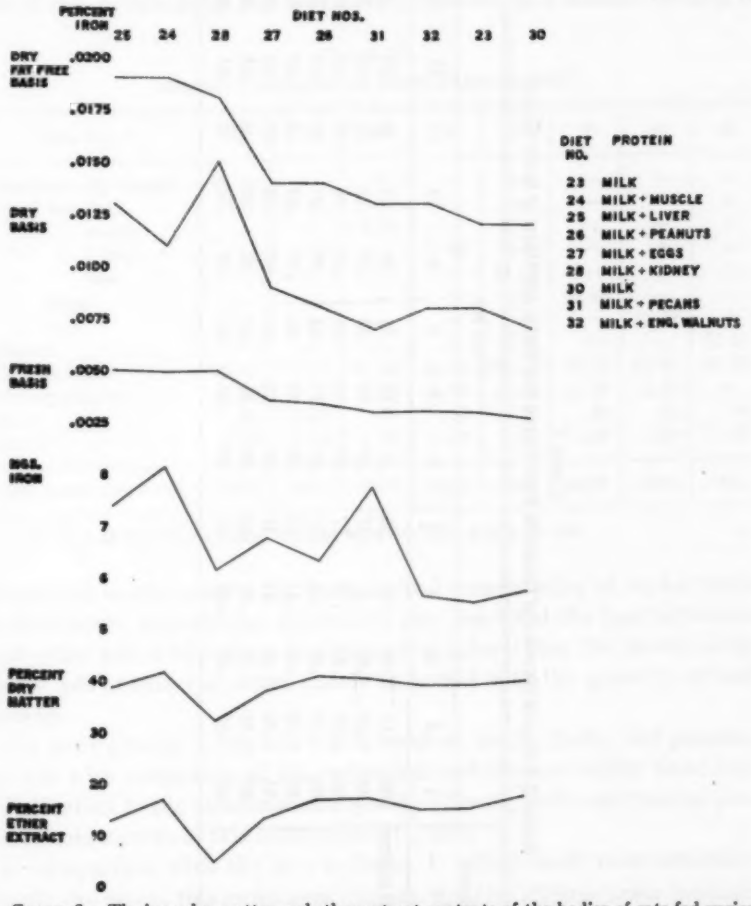

CHART 2.—The iron, dry matter and ether extract contents of the bodies of rats fed various diets.

The curves of average growth and the average gross food intakes are represented in graph No. 2, and data with respect to body gains, food intake, and the composition of the bodies of the rats are given in Table VI.

TABLE V

SERIES 2. AVERAGE NUMBER OF GRAMS OF FOOD EATEN PER WEEK, PER GRAM OF BODY WEIGHT

Diet No.	Source of protein and iron	Week No.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
33	Skimmed milk powder	.60	.55	.45	.41	.38	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24
34	Beef brain	.58	.55	.45	.41	.37	.36	.35	.33	.29	.27	.27	.25	.24	.24	.24
35	Beef muscle	.60	.55	.45	.42	.37	.36	.35	.33	.29	.28	.26	.25	.24	.24	.24
36	Beef kidney	.60	.55	.45	.41	.37	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24
37	Beef liver	.60	.55	.46	.41	.37	.36	.35	.33	.29	.27	.24	.25	.24	.24	.24
38	Beans	.53	.54	.45	.41	.37	.36	.35	.33	.29	.28	.26	.25	.24	.24	.24
39	Eggs	.60	.55	.45	.41	.36	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24
40	Peanuts	.60	.55	.45	.41	.37	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24

The best growth was obtained with the diet in which milk powder served as the sole source of protein, but good growth occurred with the rats which received beef muscle, beef kidney, beef liver, or eggs. Poor growth resulted with beef brain, beans, and peanuts, and two of the rats which received beef brain died early in the experiment. The comparative growth of the lots of rats in this series was doubtless influenced, in a manner tending to

TABLE VI

SERIES 2. COMPOSITION OF DIETS (PARTS PER 100)*

Diet; No. →	33	34	35	36	37	38	39	40
Skimmed milk powder	48.19	—	—	—	—	—	—	—
Dried beef brain	—	31.88	—	—	—	—	—	—
" " muscle	—	—	24.08	—	—	—	—	—
" " kidney	—	—	—	22.06	—	—	—	—
" " liver	—	—	—	—	22.65	—	—	—
" beans	—	—	—	—	—	64.83	—	—
" eggs	—	—	—	—	—	—	32.72	—
Peanuts	—	—	—	—	—	—	—	52.33
Corn starch	40.31	66.27	66.43	64.46	63.64	19.88	63.49	46.17
Hydrog. veg. fat	10.00	0.35	7.99	11.98	12.21	13.79	2.29	—
NaCl	.50	0.50	.50	.50	.50	.50	.50	.50
$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Iron content (per cent)	.0015	.0041	.0032	.0066	.0061	.0059	.0034	.0021

* Cod liver oil and Harris Yeast Vitamin were fed daily with each diet.

obscure the results sought, by the poor food consumption of the lot which received brain, since this lot determined and restricted the food allowance to all other lots. Under these conditions it is natural that the growth of the several lots of rats was rather closely in accord with the quantity of food allowed.

The poor growth of the lots which received beans, brain, and peanuts, and the wide variability of the individual performance within these lots, call attention to the unsatisfactory quality of bean, brain, and peanut protein as sole sources of this component of a diet.

In comparison with the rats in Series 1, which made more extensive growth, the rats in this series were characterized by slightly lower contents of dry substance, these values being lowest in the two groups (which received the bean and the brain diets) which made the poorest growth.

The growth produced by these diets is in harmony with the conclusions of Mitchell (10) that milk protein has a higher biological value than meat

protein, and that the biological values of the proteins of liver, kidney, and muscle are about equal.

The lot which received milk performed in a manner representative of the established character of this food as a poor source of iron in nutrition, the iron content of this lot of rats, on the percentage basis, being decidedly the lowest of all.

Among the lots which received liver, kidney, muscle, and eggs—the individual variation was such that it is not possible to distinguish between them with certainty. On the basis of this evidence liver, kidney, muscle, and eggs appear to be good—and nearly equally good—sources of food iron.

The absolute iron content of the bodies of the rats was highest (7 mg.) for those which received liver, was slightly lower (6.53 mgs. and 6.43 mgs.) for those which received muscle and kidney, and slightly lower than these (6.13 mgs.) for the rats which received eggs. The groups receiving beans, milk powder, brain and peanuts contained from 4.82 to 4.05 mgs. of iron, the values decreasing in the order in which the protein foods are named.

When the iron contents of the bodies are expressed as percentages of the fresh or the dry weights, the values for the milk, muscle, kidney and liver groups are in good agreement with those of the same groups in the previous work of this Institute (27); and although not perfectly comparable, they are of the same order of magnitude as the groups receiving the same foods as protein and iron supplements in Series 1 of the present paper.

The percentages of iron for the liver, muscle and kidneys groups agree closely among themselves, and are considerably higher than those for the milk group. The figures for the iron content of the milk group are but slightly higher than those reported by Supplee and associates (31) for rats receiving liquid whole milk and reconstituted dry milk. Eggs are here shown to better advantage than in the earlier work (27), and must be grouped with the meat foods as a source of dietary iron, whereas previously they appeared to have a value as a source of iron intermediate between meats and milk.

The rats which received the brain, bean, and peanut diets had percentage iron contents as high as or higher than had the meat rats. In these cases the high iron contents did not signify extensive storage of iron but were due to poor growth and to poor fattening (as indicated by low dry matter contents), the food iron therefore being concentrated as the result of the restriction of those processes which normally dilute or extend it. This is most clearly seen in the case of rat No. 582, which made the poorest growth of all. Its body content of dry matter is the lowest of the series and its percentage content of iron, on either the fresh or the dry basis, is

the highest of all. The relatively high percentages of iron in the bodies of the rats which received brain, beans, and peanuts, therefore, when considered together with the poor growth of these rats, can not be interpreted as indicating superiority of these foods as sources of food iron.

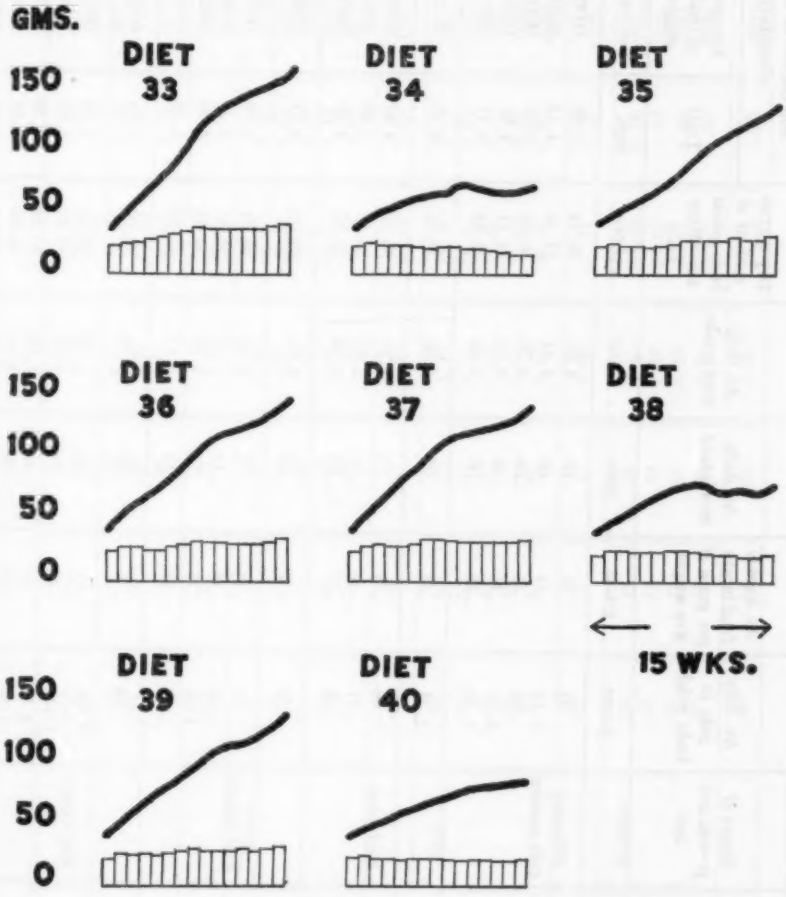

CHART 3.—The curves of average growth and the food intakes of rats fed various diets. Diet No. 33, milk as source of protein; 34, beef brain; 35, beef muscle; 36, beef kidney; 37, beef liver; 38, beans; 39, eggs; 40, peanuts.

The results of Series 2, therefore, indicate that as sole sources of food iron beef muscle, kidney, liver and eggs are superior to milk; and that beef

TABLE VII

SERIES 2. FOODS CONSUMED, GAINS IN BODY WEIGHT, AND COMPOSITION OF BODIES OF RATS

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. weekly food intake per gram of live weight	Av. daily iron intake	Av. daily food intake	Dry matter content of body minus alimentary tract	Iron content of body minus alimentary tract		
								Total	Per cent of fresh weight	Per cent of dry weight
			grams	grams	mgs.	grams	per cent	mgs.	per cent	per cent
574	33	Skimmed milk powder	.92	.35	.06	4.03	36.24	3.80	.0030	.008
575			1.16	.35	.06	4.32	32.38	4.34	.0029	.009
576			.90	.35	.06	4.20	36.05	5.05	.0041	.011
577			1.53	.35	.08	5.14	34.11	4.91	.0026	.008
578			1.74	.35	.08	5.60	33.82	6.17	.0030	.009
	Average		1.25	.35	.07	4.66	34.52	4.85	.0031	.009
581	34	Beef brain	.46	.35	.13	3.27	36.70	4.47	.0055	.015
582			.11	.34	.11	2.68	28.59	3.88	.0084	.029
583			.30	.35	.13	3.14	30.42	4.29	.0064	.021
	Average		.29	.35	.12	3.03	31.90	4.21	.0068	.022
585	35	Beef muscle	.87	.35	.11	3.42	34.31	6.56	.0054	.016
586			1.00	.35	.13	3.98	34.88	6.76	.0048	.014
587			1.01	.35	.13	4.10	34.93	6.49	.0045	.013
588			.89	.35	.12	3.85	35.82	6.30	.0050	.014
	Average		.94	.35	.12	3.84	34.99	6.53	.0049	.014
589	36	Beef kidney	.83	.35	.26	3.95	38.32	6.22	.0052	.013
590			1.05	.35	.27	4.15	35.53	6.02	.0041	.012
591			1.47	.35	.33	5.04	36.38	7.50	.0039	.011
592			1.10	.35	.30	4.57	36.01	6.88	.0044	.012
593			.54	.35	.26	3.88	33.19	5.51	.0058	.017
	Average		1.00	.35	.28	4.32	35.89	6.43	.0047	.013

592			1.10	.35	.30	4.57	36.01	6.88	.0044	.013
593			.54	.35	.26	3.88	33.19	5.51	.0058	.017
	Average		1.00	.35	.28	4.32	35.89	6.43	.0047	.013

594	37	Beef liver	.52	.35	.22	3.58	35.75	5.69	.0062	.017
595			1.09	.35	.27	4.38	35.62	6.52	.0044	.012
596			.90	.34	.26	4.25	38.02	6.88	.0053	.014
597			.91	.35	.28	4.60	39.20	7.74	.0056	.014
598			1.25	.35	.30	4.91	42.41	8.19	.0049	.012
	Average		.93	.35	.27	4.34	38.20	7.00	.0053	.014
599	38	Beans	.37	.34	.20	3.43	32.05	4.46	.0054	.017
600			.36	.35	.18	2.99	33.83	4.78	.0067	.020
601			.45	.34	.19	3.24	33.27	5.20	.0064	.019
602			.39	.34	.18	3.11	34.77	5.18	.0070	.020
603			.57	.34	.20	3.45	32.18	4.82	.0050	.015
	Average		.43	.34	.19	3.24	33.22	4.89	.0061	.018
604	39	Eggs	1.10	.35	.13	3.73	38.78	6.82	.0047	.012
605			.61	.35	.11	3.16	36.82	5.42	.0055	.015
606			1.14	.35	.14	4.14	37.14	6.28	.0041	.011
607			.69	.35	.13	3.87	36.83	5.28	.0048	.013
608			1.20	.35	.15	4.48	36.13	6.86	.0043	.012
	Average		.95	.35	.13	3.88	37.14	6.13	.0047	.013
609	40	Peanuts	.24	.35	.05	2.43	34.46	3.56	.0063	.018
610			.41	.35	.06	2.84	32.85	3.85	.0051	.016
612			.83	.35	.08	3.88	40.83	4.98	.0041	.010
613			.43	.34	.06	3.04	37.26	3.83	.0048	.013
	Average		.48	.35	.06	3.05	36.35	4.05	.0051	.014

brain, beans, and peanuts are poor sources of iron apparently because of the poor quality of their protein from a nutritional point of view.

Summary

Two series of experiments were conducted to ascertain: 1.—the relative values of beef muscle, beef liver, beef kidney, peanuts, eggs, pecans and English walnuts, as sources of iron, when each food served as a source of protein supplementary to milk powder, and 2.—the relative values of milk powder, beef muscle, beef liver, beef kidney, beef brain, eggs and peanuts as sources of iron, when each served in the diet as the sole source of protein and iron.

The experimental subjects were albino rats which were fed individually for fifteen weeks.

The best growth resulted from the diet in which the sources of protein were milk powder and pecans, though excellent growth also resulted from the diet in which milk powder was the sole source of protein. Milk powder as a source of protein, when supplemented with beef muscle, eggs, peanuts and English walnuts afforded good growth, while somewhat inferior performance resulted from milk powder and liver, and milk powder and kidney.

When serving as the sole source of protein in the diet, beef muscle, beef kidney, beef liver and eggs, all supported good growth, but were excelled in this respect by milk powder. Poor growth resulted from diets deriving their protein from beef brain, beans and peanuts.

The values of protein foods fed supplementary to milk powder, as sources of iron, were stated on the basis of absolute quantities stored, and on the basis of the contents of iron in the bodies of the rats on the fresh, the dry, and the fat-free dry basis.

On the absolute basis beef muscle, beef liver, and pecans were superior sources of iron; kidney, eggs, and peanuts were of moderate value; while English walnuts and milk powder were the poorest sources of this nutrient.

On the fresh and the fat-free dry bases beef muscle, beef liver, and beef kidney were superior sources of iron; eggs and peanuts were of moderate value; while pecans, walnuts and milk appeared to be the poorest sources of iron.

The dry body weight of the rat appeared not to be a significant basis of reference for iron contents.

As sole sources of iron, reckoned on the basis of the fresh body weights, beef muscle, beef liver, beef kidney, and eggs were superior sources of iron, while milk as usual rated poorest in this regard, the status of brain, beans,

and peanuts appearing in an unfavorable light but being questionable because of the poor or irregular growth of the rats.

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THE EFFECT OF PARTIAL DEPLETION OF VITAMIN B COMPLEX UPON LEARNING ABILITY IN RATS

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THE depletion of vitamin B complex may be either partial or complete. With complete depletion either beriberi or pellagra is developed, depending on whether the depletion is more in vitamin B (B_1) or in vitamin G(B_2) respectively. With partial depletion the usual symptom of nervous derangement may not occur in adult patients; but with children, whose nervous system is still in a stage of development, partial depletion of vitamin B complex over a long period of time might be detrimental to the proper development and function of the nervous system. That beriberi and pellagra are nerve diseases due to complete depletion of vitamin B complex is known to the public, but the detrimental effect of partial depletion has so far escaped the attention even of most physicians. Although beriberi is common in the Orient, and pellagra is prevalent in the southern states, partial depletion of vitamin B complex constitutes a world wide problem with breast and artificially fed infants and older children.

For centuries beriberi has been widespread in the Orient, and is said to have been known to the Chinese as early as 2600 B.C. The Malay States, Siam, Korea, Japan and the Philippine Islands have been greatly afflicted by it and it is more or less prevalent in India and in Africa. That it is not confined to tropical regions is shown by its occurrence in recent times in Newfoundland, Labrador and Norway. Infantile beriberi—the most accentuated form of vitamin B deficiency—is produced in breast-fed infants whose mothers partake of a diet composed mainly of polished rice. Pigeons or chickens which are restricted to a diet of milled rice develop in 2 to 4 weeks a condition of paralysis strikingly suggestive of beriberi in man. The symptoms due to derangement of the nervous system are the most spectacular, leading as they do to applying the name "polyneuritis" to the whole symptom complex.

"It should not be supposed, however, that beriberi occurs only on a rice diet. It has been shown that a number of other foodstuffs are either relatively or absolutely deficient in the beriberi-preventing vitamin. Highly milled white wheat flour and various carbohydrate foods, such as tapioca, sago and the various sugars, are quite as deficient in vitamin as rice and

when used too exclusively in the diet will produce beriberi in men or fowls with great certainty." (1)

"In point of quantity consumed white flour is the most important energy food in America and Europe. It is remarkable because it is notably deficient in more dietary factors than any other single food entering into the diet, except sugars, starches and fats which are marketed in the pure state." (2)

Furthermore, "the use of much of our food in highly refined forms has become customary in this country, and the custom is strongly fostered by the manufacturers because the refined forms are relatively immune to spoilage. So long as this custom persists, vitamin B will constitute a factor of very real practical importance in food values."(3)

McCollum points out the danger to health in an adherence to a diet in which milled cereal products, particularly white bread, and sugar, syrup, tubers and meat of the muscle type predominate. This danger is shown by the alarming increase in the incidence of defective nutrition among infants and children, brought about by the poor diet of the expectant and nursing mothers and of infants and older children.

Recent work of Goldberger and others shows that certain symptoms resembling those which occur in pellagra can be definitely traced to a deficiency of vitamin G(B_2). These symptoms are gastro-intestinal disturbance with diarrhea, degenerative disturbance of the nervous system, including mental changes, loss of weight, retarded growth and skin eruptions on the face, neck, hands and feet.

"During 1917 in an aggregated population of 22,653 individuals in some cotton-mill villages of South Carolina, 1,147 cases of pellagra (an incidence rate of 50.6 per 1,000) were observed. Of the 4,104 households among which that population was distributed 18.5 per cent had at least one member of each affected by the disease in that year. Pellagra (in an endemic locality) is very much (two to six times) more prevalent than the experience of the physicians of the locality would seem to indicate. The observation of age incidence appears to indicate that endemic pellagra is preponderatingly a disease of children of from 2 to 15 years of age." (4)

McCollum (5) is of the opinion that "improvement of the dietary is of the greatest significance for the welfare of a large percentage of American children of the present generation. Leete (1921) summarized the statistics of examination of a large part of the twenty million school children in the United States. She found . . . 15 to 25 per cent were suffering from malnutrition. Terman (1914) stated that about fourteen million of the twenty million school children in the United States were handicapped by some kind

of physical defect, and that not far from two million were suffering from a grave form of malnutrition."

The foregoing discussion may be summarized as follows: 1.—Depletion of vitamin B complex is etiologically related to such nerve diseases as beriberi, pellagra and polyneuritis, all of which involve changes in the nervous system; 2.—polished rice, the chief food supply in the Orient, and white flour bread, the main staple food in Europe and America, are both utterly deficient in vitamin B complex; and 3.—while infantile beriberi is common in the Orient, pellagra and partial depletion of vitamin B complex are found to be prevalent among the children of certain sections of the United States.

With this background in mind, one will immediately realize that the problem of vitamin B complex depletion is one of world-wide importance.

It is the purpose of our investigation to study the effect of partial depletion of vitamin B complex produced during the nursing period upon the higher nervous function or learning ability of the first and second generations of experimental animals.

We depleted our animals during the nursing period when the nervous system is still in the process of differentiation. If the depletion of vitamin B complex does produce any harmful effect on the nervous system at all, one would naturally expect to find the greatest amount of detriment when depletion is introduced during this early period.

White rats were used in our experiment because at their birth, like infants, they have nervous systems which are incompletely developed. Their life cycles are about one-thirtieth as long as that of the human. This later point enables us to study the succeeding generations, the heritage of which can easily be controlled. Finally, the anatomical and chemical analysis of the nervous system at different ages can easily be carried on with rats. Thus, in order to determine the causal relationship between diet and nervous function in a strictly scientific manner, our problems must necessarily be investigated first on small animals, of which white rats are the most practicable for our experimentation at this time. We hope to be able to repeat some of our experiments on infants in order to determine directly the applicability of our findings to human beings.

The technic used in depleting the nursing young rats of vitamin B complex merits a special description. It is well known that white rats, unlike guinea pigs, have a prolonged period of infancy. During the first three weeks after birth the young live entirely on the mother's milk. Sure (6) has demonstrated that the quantity of vitamin B in the milk of the nursing mother rat is proportional to the vitamin B content of the mother's diet.

Thus we succeeded in giving the young a diet at first deficient in and later free of vitamin B complex by merely depleting the nursing mothers of yeast and wheat germ which are the main sources of vitamin B complex. The degree of depletion was subject to variation. Some of the mothers have a larger storage of vitamin B complex than others, while the size of the litter varies from one case to another. Even in the same litter, different individuals do not receive the same amount of vitamin B complex from the mother. However, for group comparison, the technic proved to be satisfactory.

Four groups of animals were used in the present investigation. Group I consisted of 60 control animals whose mothers were fed on a normal diet rich in vitamin B complex during the nursing period. Group II, 46 in number, was depleted of vitamin B complex through the mother's diet until they were strong enough to wean. Group III, 40 in number, was raised on a normal diet rich in vitamin B complex during the nursing period although their parents, while nurslings, suffered from the depletion of vitamin B complex. Group IV, 34 rats, was depleted of vitamin B complex during the nursing period in addition to the depletion of their parents during the latter's early life.

The technic of depleting the second generation was the same as that employed in depleting the first generation. Other procedures in feeding, handling and maze training were identical for both generations.

The normal and the vitamin B depleted diets are as follows:

Constituents	Control	B-depleted
Egg albumin (extracted)	20%	20%
Salt-mixture (Osborne and Mendel)	4	4
Dextrin	29	29
Starch	23	35
Agar	2	2
Butter	5	5
Cod liver oil	5	5
Yeast	7	0
Wheat embryo	5	0

Egg albumin, dextrin and starch are all extracted free of vitamin B complex. Preliminary work several years ago led us to include yeast and wheat embryo in the normal diet so that both B(B_1) and G(B_2) are present.

To repeat, our animals were depleted only during the nursing period. After the depleted animals were weaned, they were given a week of vitamin-rich diet so that their body weight was brought nearer to that of the control animals. On the other hand, when the control animals were weaned they were fed on the B-free diet for a few days, just enough to check their

rate of growth without seriously depleting them. From 49 days of age on, each group was placed on exactly the same diet which consisted of Sure's B-free food plus brewer's yeast.

As soon as the animals reached the age of 70 days, they were given a preliminary training in the food box of a standard maze once a day for a week. This was done to inculcate in the white rats the association between maze and food satisfaction, as well as to familiarize them with the general experimental situation. The maze we used contained nine culs-de-sac or blind-alleys and was one of considerable difficulty. It was designed by Professor Harvey Carr of the Psychology Department of the University of Chicago and has been widely used as a standard apparatus in the field of comparative psychology.

After the week of preliminary training, the animals were required to run the maze once daily during the first three days; after this two trials were given a day until the problem was learned. The criterion of learning was 8 correct out of 10 consecutive trials.

The incentive used was a freshly dealcoholized vitamin concentrate[1] from the wheat germ. Sure's B-free food plus brewer's yeast, available with water, was always in the living cage of each animal after the age of 49 days. Thus extreme hunger was avoided. The animals, however, were eager for the incentive used. Prelimi-

[1] Wheatamin.

TABLE I

VITAMIN B COMPLEX AND MAZE LEARNING OF FIRST AND SECOND GENERATIONS

Group	No. of rats	Av. weights during maze learning		Diet while nursling		Average Maze Scores						
				Generation		Trials			Errors			Time
		Start Maze	Finish Maze	First	Second	Av.	St. D	PE	Av.	St. D	PE	Sec.
I	60	131	181	B Comp. Rich		45	30	2.6	108	66	6.7	1992
II	46	96	173	B " Depl.		106	80	6.9	248	175	17.3	4210
III	40	123	158	B " "	B Comp. Rich	43	21	2.3	122	64	6.7	2093
IV	34	83	161	B " "	B " Depl.	106	86	9.8	245	143	16.5	4120

St. D—Standard deviation. PE—Probable error.

nary work showed that this represented the best procedure in getting the groups to the nearest approximation in their relative strengths of incentive.

The results thus obtained in maze learning are to be compared according to each of the three criteria of measurement; the number of trials before mastery, the number of cul-de-sac errors, and the length of time in seconds required for running the maze.

The comparative average scores of the four groups in maze learning are presented in Table I.

The results may be summarized as follows:

1.—As to the first generation, those animals which had been depleted of vitamin B complex through their mothers' diet during the nursing period are very much inferior in learning ability to the normal animals which received a vitamin rich diet while they were nurslings. The differences are very large and consistent with all the criteria of measurement. Normal rats learn about twice as efficiently as the vitamin B-depleted animals. These data confirm our previous findings with smaller groups.

2.—As to the second generation, namely, the offspring of rats depleted of vitamin B complex in their early life, those animals which were again, like their parents, depleted of vitamin B complex during the nursing period are very much inferior in learning ability to those which, unlike their parents, received a vitamin B-rich diet while they were nurslings. The differences are again very large and consistent with all the criteria of measurement. Vitamin B-rich animals from depleted parents learn about twice as efficiently as vitamin B-depleted animals from depleted parents. These data of the second generation confirm our findings of the first generation.

3.—When the offspring of the depleted animals are brought up on a vitamin B-rich diet, their learning ability is practically equal to that of the normal animals. This seems to suggest that the learning ability of the second generation is not affected by the early depletion of vitamin B complex on the part of the first generation.[2] This may mean that the detrimental effect of vitamin B deficiency upon learning ability is not transmitted to the offspring through the germ plasm. At any rate, the data clearly indicate the possibility of restoring the learning ability of the offspring to a normal level by giving them a normal diet during the nursing period.

[2] There is perhaps a selective factor here as the infant mortality is much higher. See Author's article on Infant Mortality, to be published shortly.

4.—When the offspring of the depleted animals are depleted of vitamin B complex during the nursing period, their learning ability is practically equal to that of the depleted parents. Here again, it suggests that the detrimental effect is not transmitted from the parents to the offspring as the effect is not cumulative through generations. Further work is being done with the third and fourth generations.

5.—The comparison in maze records between the vitamin B-depleted animals and their own offspring brought up on a vitamin B-rich diet proves conclusively that the large differences in their learning ability are not due to the genetic factor of family strain, but to the dietary factor of vitamin B depletion. These data confirm our previous findings obtained by comparing depleted and control litters of the same parentage.

In short, our data prove conclusively that early partial depletion of vitamin B complex is detrimental to learning ability but that the offspring of depleted animals can attain the normal level of learning ability provided they are brought up on a vitamin B-rich diet during the nursing period.

Having established the causal relationship between dietary deficiency and learning ability in experimental animals, we shall consider the applicability of our findings to human beings.

Let us first cite the work of McCollum, Parsons and Kalmbach (7). "In 1919 we sought an opportunity to test on human beings to what extent the principles established in animal experiments apply to the nutrition of children. We sought a group of children who, because of poverty or lack of knowledge on the part of those responsible for their care, were being fed a diet which we suspected to be inadequate for the production of growth and well-being. We discovered an institution in which there were 236 negro children ranging in age from early infancy to twelve years, whose environment and food supply left little to be desired in relation to our objective. Severe malnutrition existed in most of them; it appeared that the cause was probably inadequacy of their diet. An opportunity was offered to improve the quality of their diet in order to observe the extent to which the children would respond to growth and well-being.

"The diet of these children during the period when growth should be proceeding at a rapid rate consisted essentially of cereals, tubers, fleshy roots and muscle meats, and a considerable portion of the food was refined wheat flour in the form of white bread.

"The simplest procedure for improving the quality of this diet without involving additional cookery appeared to be the addition of milk. One group of 42 children received 1 quart of whole milk each per day during

the demonstration period. This was prepared from whole milk powder,[8] by dissolving the proper amount of the whole milk powder in cold water and agitating in a mixer. The other group, designated the check group, was continued on the institutional diet.

"There was a marked change in the behavior of the milk-fed group as contrasted with the check group. The latter were apathetic and very tractable. The discipline of the institution was strict and these children were all obedient. Those in the milk-fed group, on the other hand, soon caused annoyance to their teachers by their restlessness and desires for activity, and were frequently guilty of infractions of the rules."

Next we shall cite Blanton's work (8) in the war zone: While located with the Army of Occupation in Trier, Germany, Blanton had opportunity to study the effects of prolonged under nutrition on the nervous systems and the mentality of 6,500 German school children. Because of the many complaints of the teachers regarding the mental deterioration and the increase in nervous disorders, Blanton undertook a detailed study of the situation. The children were between five and a half and fourteen years of age.

"At least forty per cent of the children in the Volksschulen of Trier, Germany, are suffering from malnutrition to such a degree as to cause a loss of nervous energy. The percentage of children failing to pass their grades has increased from an average of eight per cent in pre-war years to fifteen per cent in 1917 and 1918. It is estimated about half of this seven per cent increase in retardation has been due to malnutrition, the other half has been due to war conditions. There has been a lowering of the whole standard of school work caused chiefly by malnutrition but partly by war conditions in general; half of the children who in pre-war time did superior work now do average work, and the percentage of children who did inferior work has been increased from twenty to more than thirty per cent. The specific changes noted in the children, caused by malnutrition, are: (a) lack of nervous and physical energy, (b) inattention during school hours, (c) poor and slow comprehension for school tasks, (d) poor memory for school work, and (e) a general nervous restlessness while in school."

Finally, we shall cite the work of Hoefer and Hardy (9). These investigators reported the results of tests and measurements made on 383 children, ranging in ages from 7 to 13 years and grouped according to the length of the period of exclusive breast feeding.

"Mental development was measured by individual psychologic examinations with the Stanford Revision of the Binet-Simon intelligence

[8] Klim.

test and the Pintner-Patterson performance scale (a nonverbal intelligence test), and by a group educational test, the Stanford achievement test. The results for these tests are recorded in terms of quotients which express the relationship between the child's ability and what is expected of his age.

"From an analysis of the results on mental development, it will be seen that the artificially fed were inferior in all standardized measurements to those breast fed from four to nine months, and, with one exception, to those breast fed three months or less. They, however, equaled or excelled those breast fed from ten to twenty months. In learning to talk, the artificially fed were the most retarded of all the groups. The importance of breast feeding is further emphasized by the fact that among those children having the highest intelligence quotients, not a child artificially fed rated as high as 130, while 5 per cent of those breast fed were above this score."

In the light of our experimental data, these investigations on children, although suggestive in character, seem to indicate that the relationship between dietary deficiency and higher nervous function also exists in human beings.

The next problem was raised by Barnett Sure (10) as follows: "Our illuminating findings that nursing young of the albino rat need approximately 100 times as much of vitamin B (calculated in terms of dehydrated yeast) as vitamins A and D (as furnished by a brand of cod liver oil employed by us since 1921) for growth during the nursing period brings up the question: Do infants, breast or artificially fed, receive adequate amounts of vitamin B?"

"The usual dietary of an infant in the United States, up to its third month, consists of human milk or cow's milk, to which has been added a sugar or a syrup and possibly a milled cereal, with some orange juice and cod liver oil. When this diet is analyzed for its vitamin content, A, C and D are found to be present, but vitamin B is represented only in milk and in orange juice, and in these only in limited amounts. From the foregoing discussion it is plain that only minimal, and in some circumstances subminimal, amounts of vitamin B are to be found in such a dietary (11).

In view of the widespread chronic partial deficiency of vitamin B complex in Asia, Europe and America, not to mention Australia and Africa, due to the large consumption of polished rice and highly milled white flour bread, it is an urgent necessity that vitamin B complex should be supplemented in sufficient quantity to the diet of the expectant and

nursing mothers as well as to that of the breast and artificially fed infants and older children.

To summarize, our data on experimental animals have proved conclusively that early partial depletion of vitamin B complex is detrimental to higher nervous function as measured by maze learning ability; but that the offspring of the depleted animals, *i.e.*, those that survive the high infant mortality, can attain the normal level of learning ability provided they are brought up on a diet rich in vitamin B complex.

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THE EFFECTS OF RADIANT ENERGY ON MILK ANEMIA IN RATS*

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FROM a review of the literature it may be concluded that normal men and animals on an ample, balanced diet are able to live under conditions in which sunlight or artificial radiation is entirely absent or much reduced for long periods of time, perhaps indefinitely, with no resulting anemia. Conversely, increasing the amount of radiation which a man or animal receives, up to a limit that does not produce harm, does not produce changes in the blood picture other than to alter the blood volume temporarily. On the other hand, in the diseased organism there is considerable evidence that radiation often results in improvement of the blood picture. The older experimental work on this subject is unsatisfactory and contradictory, see the reviews by Reed (1925), Hardy (1927) and Laurens (1928). There was no standardization of the source of radiation; in fact, no appreciation of what the source was really emitting. Such work naturally cannot be repeated.

Osato and Tanaka (1929) report that quartz mercury arc radiation mobilizes the iron reserve in the spleen and liver and hastens hemoglobin regeneration in dogs, rabbits and rats made anemic by venesection. Laurens and Mayerson (1931) have just shown that, although carbon and mercury arc irradiation results in a marked and persistent increase in the number of erythrocytes and reticulocytes in hemorrhagic anemia, such treatment fails to accelerate the regeneration of hemoglobin. In acetylphenylhydrazine (pyrodin) anemia, on the other hand, Mayerson and Laurens (1931) found that such radiant energy, although it had no effect on the downward course of the anemia, hastened the regeneration of hemoglobin as well as that of the red cells.

The effects of radiation on a nutritional type of anemia have received

* The data in this paper are taken from a thesis submitted to the Faculty of the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1930. The investigation was carried out under the direction of Professor Henry Laurens.

Preliminary reports on the results have appeared in the *Proceedings of the Society for Experimental Biology and Medicine*, 1929, xxvi, 251; 1930, xxvii, 892.

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little experimental study. Mathews, Doyle and Whiting (1929) irradiated pigs with the quartz mercury lamp and with sunshine in an attempt to benefit a naturally occurring anemia. Little benefit as regards the anemia was noted in the pigs irradiated with the quartz lamp although the mortality of this group was lower than in the non-irradiated pigs. Young growing pigs kept in the sunshine showed at the end of 38 days hemoglobin levels which were higher than in control animals. Hart and co-workers (1927) noted no beneficial effects of mercury arc radiation on the blood of rabbits rendered anemic by a milk diet. These observations were incidental to another problem and apparently not based on a large series of results. It is reasonable to inquire whether or not radiant energy would be effective in promoting the metabolism of iron when that substance is low in the diet. Accordingly, it was decided to produce anemia in rats by feeding them an iron-poor diet (milk) and to observe the effect of radiation in preventing or curing this anemia.

The experimental procedure was simple. Rats from a healthy albino strain bred in our own laboratory, were divided into groups of about 10 each, when 21 to 28 days old, care being taken to keep litter and sex distribution equal. Some of the groups were exposed to varying amounts of radiant energy; others receiving no irradiation served as one group of controls. Other groups of controls consisted of rats on the stock diet receiving no radiation and of rats on the stock diet receiving radiation. The observations were continued for from 10 to 14 weeks. The stock diet was a commercial meal (Maynard, 1930) supplemented by lettuce twice a week. Powdered whole milk[1] was used in preparing the milk diet. By purchasing large quantities of the powder at a time from the same lot, a uniform diet was insured over a long period of time, an advantage which we could not obtain by the use of our available source of dairy milk. Solutions containing 12.5 per cent in distilled water were fed *ad libitum*. Such solutions, according to the manufacturers, have approximately the same composition as fresh dairy milk. The drinking water supplied was distilled. Care was taken to prevent the transference of food material or excreta from one cage to another.

Hemoglobin determinations were made once each week on blood from the tail with a Newcomer instrument standardized by oxygen capacity determinations with the Van Slyke manometric apparatus. The rats were weighed weekly. Red and white counts and red cell volume readings (hematocrit) were made at the end of, and once or twice during, each

[1] Klim.

experiment. Neubauer chambers were used for the cell counts and Van Allen tubes for the hematocrits. From the values obtained for the hemoglobin, red cell counts and volume, the average corpuscular volume and saturation were calculated. The use of such figures permits the expression of the data relating to the red cell in absolute values rather than in terms of "indices" or "coefficients."

The sources of radiant energy used were a "Pan-Ray-Arc"[1] burning National Therapeutic A "Sunshine" carbons and an air cooled Cooper-Hewitt quartz mercury vapor lamp. The carbon arc as operated emits an average of 0.820 gm.-cal. per sq. cm. per min. of energy incident at 1 meter and has a distribution of 5 to 6 per cent ultra violet, 29 to 31 per cent luminous and 63 to 66 per cent infra red. The mercury lamp emitted 0.0619 gm.-cal. per sq. cm. per min. incident at 1 meter distributed as follows: 13 per cent ultra violet, 7 per cent luminous (400–1400 mμ), and 80 per cent infra red. Further details are given in the papers by Laurens and Mayerson (1929 and 1931).

The hemoglobin of the rats at birth was about 14 gms. per cent. At 3 weeks of age, when they were weaned, it had dropped to 8 or 9 gms. per cent. When rats are placed on a milk diet the hemoglobin continues to decline, dropping to about 5 gms. per cent in from 4 to 6 weeks and many of the animals die. In those that survive the hemoglobin increases to about 6 gms. per cent and the rats live on with their hemoglobin at this value. When placed on the standard calf meal diet at weaning, the hemoglobin immediately begins to rise and reaches about 15 gms. per cent at the end of 10 weeks. Growth on the milk diet was not, as a rule, greatly retarded during the first 2 to 4 weeks, but by the end of 10 to 12 weeks such rats weighed only 140 to 160 grams as compared with 190 to 240 grams for rats reared on the calf meal. When first placed on the milk diet rats almost universally develop a severe diarrhea, large quantities of mucus being sometimes observed in the feces. As above stated the death rate is high. Gross examination fails to reveal intestinal lesions. The diarrhea disappears at about the same time that the slight, spontaneous rise in hemoglobin takes place. Perhaps the intestinal disturbance is a factor in the genesis of the anemia, preventing adequate absorption of the small amounts of blood-building material in the diet. The fall in hemoglobin is most rapid during the first 4 to 6 weeks on the milk diet which is the period of most rapid growth. During the following weeks growth is considerably slower and consequently there is a balance struck between

[1] Made by Atlas Electric Devices Co.

the requirements for and the intake of hemoglobin-building substance. Rats on the milk diet and growing abnormally slowly, maintained higher hemoglobin concentrations than did rats growing rapidly. None of the rats showed evidences of rickets.

At the end of some of the observational periods the female rats were placed on the stock diet and subsequently mated with males that had been reared on this. In this way many litters were obtained and designated as "second-generation rats." These when weaned and placed on the milk diet, became anemic in a slightly shorter time and showed less tendency to recover spontaneously than did rats from the stock colony, although the lowest levels reached were about the same in both groups. Similarly "second-generation" rats were placed on the stock diet and mated with healthy males giving litters which were designated as "third-generation rats." Beyond the third generation the breeding propensities became so poor that it was not possible to continue in this manner.

The red cells of the anemic rats showed great variation in size and shape, both of which features are characteristic of secondary anemia and may be taken as one index of its severity. The cells were much below the average in size (see Table I), the size tending to vary inversely with the severity of the anemia. About 15 per cent of the cells in the anemic bloods

THE EFFECT OF RADIANT ENERGY ON MILK ANEMIA, EXPERIMENT 16. AVERAGES OF GROUPS OF ABOUT 10 EACH. VALUES OBTAINED IN THE 10TH WEEK.

Conditions	Weight gms.	Hb level gms. per cent	Hemato-crit per cent	R.B.C. millions	Corp. vol. cu. μ	Corp. Hb gms. $\times 10^{-12}$	Satura-tion per cent
1. Stock diet No irradiation	216	16.3	42.0	8.23	51.0	19.8	38.8
2. Milk diet No irradiation	140	6.1	19.5	6.68	29.2	9.1	31.3
3. Milk diet, Hg arc 5 min. daily	160	11.3	34.5	7.70	44.8	14.7	32.8
4. Milk diet, Hg arc 15 min. semi-weekly	163	13.0	35.0	7.84	44.6	16.6	37.1
5. Milk diet, Hg arc 15 min. daily	168	12.4	33.0	8.52	38.8	14.6	37.6

were polychromatophylic. The hemoglobin content of the cells was low and distributed about the periphery of the cells leaving the central portion clear. The red cell counts were not much lower in the anemic than in the normal rats. The resistance of the red cells to hypotonic solutions was

markedly increased. Total white cell counts in the anemic animals were lower than in the normal ones, this being due chiefly to diminution in lymphocytes, the neutrophiles being unchanged or increased.

The hemoglobin values in 11 of 18 groups of rats given various amounts of quartz mercury arc radiation were definitely higher than in the controls, slightly higher in 4, the same in 1, and lower in 2 groups than in the controls. The periods of irradiation varied from 4 minutes to 25 minutes daily and from 15 minutes to 35 minutes semi-weekly. In some experiments the irradiation was begun when the rats were first placed on milk, in others not until after the hemoglobin had dropped to low levels. The results of some of the best experiments are given in Figure 1. In general, the radiant energy failed to prevent the initial fall in hemoglobin al-

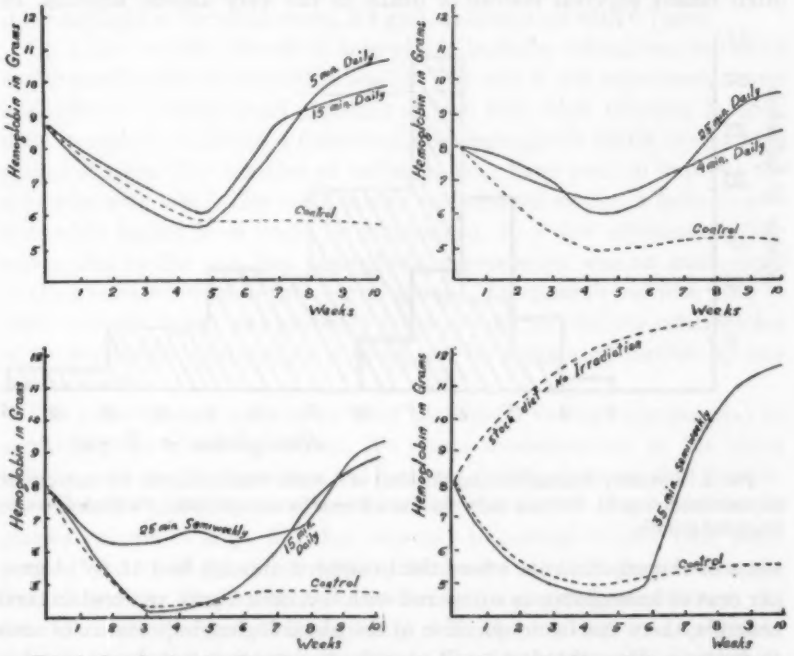

FIG. 1. Graphs showing the effect of mercury arc radiation on the hemoglobin content of rats maintained on a milk diet. Each curve represents the averages of a group of 7 to 10 individuals. In each of these experiments the radiation was given throughout the 10 weeks' period.

though in several series this fall was retarded and diminished. It was after the fourth week, when the hemoglobin had reached its lowest level, that the effect was greatest. Not only was an increase in the hemoglobin con-

tent of the blood noticed, but the red cells became more normal in their number, saturation, size, shape and staining. The results of a typical experiment are shown in the table (p. 520). Small doses (4 to 5 minutes daily) were in general as effective as larger doses (15 to 25 minutes daily). Growth was usually improved by small or moderate doses, but there was a tendency for larger doses to inhibit it. The mortality in general was lower in the irradiated than in the non-irradiated groups. The effect on the white cells was inconstant.

The differences in hemoglobin between the irradiated and non-irradiated groups were not large but the weighted mean value for all animals surviving 10 weeks or longer is 8.29 ± 0.130 gms. in irradiated animals, and 5.80 ± 0.096 gms. in the controls (Fig. 2). The difference, as small as it is, often meant survival instead of death in the very anemic animals. In

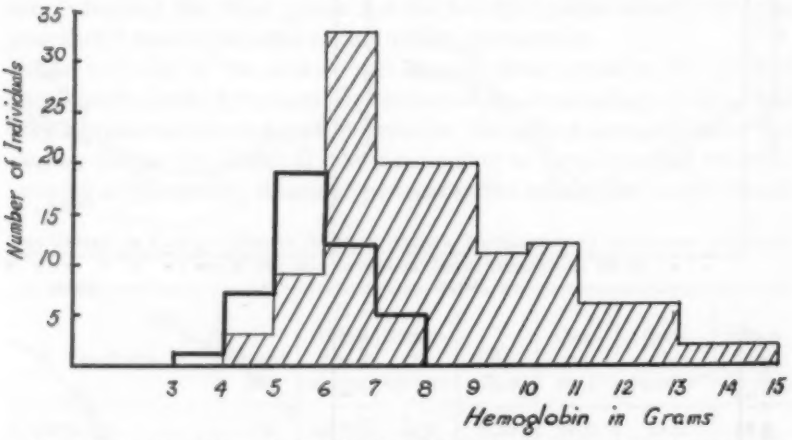

FIG. 2. Frequency diagram showing the effect of 8 weeks' irradiation with the mercury arc on hemoglobin content. The area under the heavy lines is for non-irradiated, the shaded area for irradiated animals.

some of the experiments where the irradiated animals had 11 to 13 gms. per cent of hemoglobin as compared with less than 6 gms. per cent in their controls, there can be no question of the physiological importance of such an increase. Here the sleek, well nourished, active irradiated rats stood in marked contrast to the scrawny, emaciated, inactive controls.

Irradiation with the flaming carbon arc resulted negatively. Of 15 groups, the hemoglobin values in 8 were equal to or lower than their controls, in 7 slightly higher, but in only one group sufficiently so to be indicative, 7.9 gms. as compared with 5.7 gms. in the control. The energy

from the carbon arc was given in a wide variety of doses, varying from 5 minutes to an hour daily and 5 minutes to an hour semi-weekly at a distance of 1 meter. The reason for the effectiveness of the mercury lamp as contrasted with the ineffectiveness of the carbon lamp is not apparent, although it seems clear that the difference between the activity of the two sources was not dependent upon the total amount of energy. It would be unwarranted to conclude that the carbon arc has no beneficial results on the blood picture, but certainly under the conditions of our experiments no such effects were noted.

A group of 16 rats exposed to sunshine for a half hour daily, beginning at 10 o'clock, through June and July, had hemoglobin values at the end of 7 weeks slightly higher than in 9 control rats exposed only to the subdued daylight of the stock room, 8.4 gms. as compared with 6.7 gms.

On a diet entirely devoid of hemoglobin-building substances, radiation could hardly produce favorable results. The rats in our experiments were undoubtedly getting small amounts of iron and other minerals in their diet, enough to maintain a balance at low hemoglobin levels over a long period of time. The function of radiation may have been to improve the economy with which this small intake was utilized so that a balance at a somewhat higher level could be maintained. In a few experiments iron was added to the diet, but hemoglobin regeneration was no more rapid in the irradiated than in the control groups. The quantity of iron used (1 mgm. per rat daily) was probably large enough to produce regeneration at its maximum rate so that it could not be stimulated further by any other means.

The question may arise as to what part blood volume changes play in explaining the observed results. No direct measurements of the blood volume were made. Mayerson and Laurens (1928) observed that irradiation with the carbon arc resulted in an increase of 6 to 37 per cent in the plasma volume of dogs, but that recovery to normal volume took place within 5 hours. Most of our observations were made 24 hours after the last irradiation. The fact that an increase in the size and saturation of the red cells was observed concomitantly with the increase in hemoglobin argues against the changes being due to blood concentration. Further, it is hardly conceivable that blood volume changes could bring the hemoglobin values from 5 or 6 grams to 12 or 13 grams as in some of our experiments. Moreover, the hemoglobin values were practically the same whether the determinations were made one day or several days after the last period of irradiation. The results are also not explained on the basis of inhibition of growth since some of the animals which showed the best

hematopoietic response also showed marked acceleration in the rate of growth, and, contrariwise, some whose growth was inhibited, failed to show increased hemoglobin concentration.

In all probability the radiation, when effective, stimulated body activity, bringing about improvement in the general nutritional state, and this is reflected in part in the improvement in the blood picture.

Summary

Irradiation with a quartz mercury arc has a slight but definite effect in increasing the hemoglobin content of the blood of rats rendered anemic by a milk diet. The number, size, and saturation of the red cells are also increased. A flaming carbon arc, from "Sunshine" carbons, had no effect.

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Editorial Review

SOME ESSENTIALS OF A GOOD NUTRITION EXPERIMENT

A PERFECT experiment in any field of science may be said to be one that has been planned and conducted in such a way that the results obtained are susceptible of only one interpretation. Only such an experiment can amount to a demonstration. To the extent that this ideal has not been realized, in that more than one interpretation of the results is possible, to that extent the experiment has established only a certain limited probability in favor of the conclusion drawn. But this requirement of a perfect experiment is difficult to realize, particularly in the biological sciences. Two illustrations, one in a physical science and one in the science of nutrition, may serve to emphasize the difficulties.

In 1887 the famous Michelson-Morley experiments were undertaken to measure the velocity of the earth through the ether. The general principle upon which these experiments were based was that the velocity of light, as observed on the earth, would depend upon the direction of the light relative to the direction of the earth's motion through the ether, the extent of this dependence affording a measure of the earth's velocity. The technic of the experiments need not be described here except to point out that the apparatus used was so sensitive that a velocity of the earth as slow as 1 meter per second could be detected. The experiments were repeated a number of times but always with the same result, namely, that the velocity of light was quite independent of its direction. Hence, the earth appeared to be stationary in the ether. Here is an experiment, performed with the utmost precision, which seemed susceptible of only one interpretation. However, the interpretation is quite inconsistent with the known fact that the earth is traveling around the sun at a speed of nearly twenty miles a second. In such a situation, there must be an alternative explanation of the experimental results. An alternative explanation was later presented. It was shown, on the basis of the electrodynamical theory then accepted, that bodies moving through ether are contracted in the direction of their motion, and that in the Michelson-Morley experiments the differential contraction of the apparatus, and in particular the contraction of the measured paths over which the light traveled, may have been just sufficient to compensate for the differences in the velocity of light induced by differences in its direction. However, other methods of measuring the effect of direction upon the velocity of light have also given negative results, al-

though these methods have not involved the use of material measuring rods, so that the alternative explanation of the Michelson-Morley experiments does not appear to embody the whole truth. Hence, what appeared to be a precise experiment, capable of giving results susceptible of only one interpretation, seems, in fact, quite uninterpretable at the present time. In the words of Sir James Jeans: "It appeared then that if the earth moved through the ether this motion was concealed by a universal shrinkage of matter, and this shrinkage was in turn concealed by some other agency or agencies whose wit, so far, appeared to be greater than that of man."

In the physical sciences experimental conditions may be much more rigorously controlled than in the biological sciences. Furthermore, the problems in the physical sciences are so much more often open to direct attack. It is to be expected, therefore, that a perfect experiment would be more difficult to realize in the biological sciences than in the physical sciences. Let us consider a well-controlled nutrition experiment concerned, let us say, with the question as to whether or not a certain amino acid is indispensable for growth. A ration is prepared including a low percentage of a protein known to contain only traces of the amino acid in question, but including in adequate concentrations all other essential growth factors, in so far as current knowledge of such factors extends. Another ration is prepared, similar to the first except that a small percentage of the amino acid is substituted for an equal percentage of starch. Two groups of comparable experimental animals are now selected, one group to receive the first ration and the other the second. To make the experiment more directly and easily interpretable in terms of the relative growth-promoting values of the two rations, the animals are fed individually and the amounts consumed are regulated in each case so that the rates of gain are the same, within narrow limits, for all animals. After a feeding period of several weeks duration, the total intake of food is computed for each animal. It is found that less food was required by all of the animals on the second (supplemented) ration than by any of the animals on the control ration to produce the same increase in body weight. The conclusion seems clear that the amino acid included in the second ration was indispensable for growth.

However, there are other possible interpretations of this experiment, no one of which can be ruled out on the basis of the data obtained:

1. The gains in weight in the two groups of animals may differ in composition. If the gains induced by the supplemented ration contained more water or more fat or both than the gains induced by the control ration, the former ration may be no more efficient than the latter in the promotion of growth, or more specifically, in the promotion of protein synthesis.

2. The basal metabolism of the experimental animals may have been depressed by the added amino acid, in which case less food would be required to produce a given gain in weight because a greater proportion of the food eaten would be available for this purpose.

3. The activity of the animals may have been depressed by the added amino acid, again leaving more food available for growth. The effect of different rations upon the voluntary activity of experimental animals is almost a virgin field of investigation.

4. The added amino acid may be serving as a precursor for some indispensable dietary constituent (vitamin) not at present known or characterized and not contained in adequate amount in the basal ration. Its function in this case may be quite unassociated with protein synthesis.

It may be objected to this analysis of the experiment that the alternative explanations are quite improbable, even though not inconceivable. The objection cannot be over-ruled, but it may be pointed out that any assessment of the probability of an event is based only upon current knowledge, which may be seriously incomplete. The progress of science inevitably changes the probability of the occurrence of events. What seems improbable today becomes quite probable tomorrow, or it may even be established with certainty. I think it must be admitted that the experiment under discussion does not *demonstrate* anything in any strict sense of the term simply because its results are susceptible to a number of possible interpretations, although the most obvious interpretation *seems* to be far more probable than the others.

The same analysis could be made of any experiment in which the relation between dietary constituents and the rate of increase in body weight is being established. This is true either because the composition of gains in weight depends in part upon the composition of the diet consumed, or because the food available for increase in weight is the difference between the total food consumed, which may be controlled, and the food used for the maintenance of life and for voluntary activities, which can be only incompletely controlled by the investigator.

The situation discussed above seems typical of experimentation in nutrition in so far as it relates to the difficulties of interpretation. It impels caution in the formulation of conclusions on the one hand, and on the other hand it stresses the importance of a precise technic and of a rigorous control of experimental conditions, since the most rigorous control will not remove all of the uncertainties of interpretation. Over-confidence in the most obvious or the most probable interpretation of an experiment and a corresponding over-positiveness in its formulation may perpetuate a mis-

conception and seriously impede scientific progress, while excessive caution will at most delay temporarily the establishment of a truth.

While it may not be possible with many types of nutrition problems to demonstrate anything in the strictest sense of the term in a single experiment, because of a multiplicity of possible interpretations, it is sound policy to reduce the number of possible interpretations to a minimum. This can be done only by extending control in so far as feasible to every experimental condition that could possibly influence the animal function under observation and measurement, so that control and test animals are subjected in an equal degree to all experimental conditions except the one being studied, or that, in control and test periods, experimental conditions are similarly equalized. It is, of course, impossible to consider in this paper all of the implications of this proposition. Attention will be confined, therefore, to only a few of them.

It is generally recognized that the problems of nutrition are essentially statistical in nature in the sense that they require an accumulation of data for their solution. Experimental animals, no matter how carefully they have been selected, exhibit variability in functioning under the same conditions of treatment. Hence, experiments involving only one or two or three animals can only rarely be expected to furnish a decisive answer to an experimental inquiry. It is a common practice to include a larger number of animals on each experimental treatment, though unfortunately there is no common agreement either on the precise number to use or on the logical method to use in determining in each case how many may be necessary. Statistical methods can aid in avoiding, on the one hand, the embarrassment attending the conclusion of an experiment that has yielded such a small amount of variable data that no definite interpretation is possible, and, on the other hand, the quite unnecessary labor and expense in obtaining far more data than are required to clinch the point at issue. But unfortunately statistical methods are but slowly coming into vogue in nutrition laboratories.

After deciding, arbitrarily or logically, how many animals are to be employed, one must next decide whether to feed them in groups or individually. Both methods are quite commonly followed. It seems well, therefore, to consider which is the best. Group feeding is the more economical of labor. This is perhaps its greatest if not its only advantage over the individual feeding method. If it possessed no disadvantages, the saving of labor, or the ability to carry with the same amount of labor a larger number of experimental animals would be decisive, but if the saving of labor can be secured only by a serious sacrifice in precision, because of a less complete

control of the experiment, then it should not be accorded any considerable importance.

Another advantage of the group-feeding method that is urged particularly in livestock experimentation or in experimentation concerned more or less directly with the practical problems of livestock farming, is that it is a more natural method of feeding, involving no restriction on appetite except that imposed by the competition of animals within the group. However, natural conditions mean uncontrolled conditions and the prevalence of uncontrolled conditions means ineffective experimentation. If science were compelled to forego entirely the control of conditions in its seeking for truth, and to make its observations only under those conditions of living "natural" to the species of animals being observed, it would have to dispense entirely with the experimental method. While nobody would perhaps take this extreme position, there are apparently many who hesitate at each step in the imposition of experimental control for fear that the further one proceeds from the "natural," the less applicable the observations secured become to animals in their "natural" environment. This attitude leads to a compromise somewhere in the planning of the experiment between the demands of good experimentation and the fear of the effects of limiting the "natural," or the "instinctive," behavior of experimental animals.

However, it seems a fair judgment that this fear is a baseless one unless there is some objective evidence that a given experimental procedure will yield spurious results of no general applicability, or unless there is some good logic leading to the belief that such would be the case. In attacking the overwhelming majority of problems in the science of nutrition it seems a greater evil to compromise with the demands of good experimentation than to risk the possibility, generally remote, that some of these demands may limit the usefulness of the evidence obtained. In the former case all too frequently the evidence obtained is too vague for any sure application whatsoever; in the latter case, at the very least, the stage has been set for the production of a definite contribution to knowledge.

The disadvantages of the group-feeding method are serious. In applying this method, the investigator removes the possibility of measuring the food intakes of individual animals and also of controlling them. If, as in livestock experimentation, he is concerned with measuring the amount of food required to produce a unit of gain in body weight, he has deprived himself of all means of measuring the experimental error of the average value of this ratio. An average entirely dissociated from the individual items of which it is composed is a weapon of dubious value in attacking

scientific problems. It may be as good as its face value (or nearly as good), or its effectiveness may be seriously impaired by a large experimental error. Whether the former is true or the latter can be decided only by consulting the individual items, whose number and variability will determine the experimental error of the mean result. The difference in significance between a group of widely variable data and another group possessing the same mean value but a much smaller variability is analogous to the difference in climate between two localities possessing equal mean annual temperatures of 70°F., but mean ranges in one case from −20°F. to 105°F., and in the other case from 35°F. to 85°F. To say that the climates are the same because the mean annual temperatures are the same is seriously to distort the truth.

Any measurement made on the individual animals of a group, be it rate of gain in weight, blood composition, bone composition, or the like, will presumably be affected by the amount of food consumed unless definite evidence to the contrary is at hand. The assumption, for example, that the extent of calcification of the bones is not affected by a variable consumption of a ration possessing a constant content of vitamin D and a constant calcium-to-phosphorus ratio, is indefensible until it has been established by direct experiment. The variability in these measurements within experimental groups fed together may be due to a considerable extent to a variable intake of food. But whether this is true and to what extent it is true cannot be told with no records of individual consumption available. The variability in the measurement cannot be interpreted with reference to the intakes of food, it cannot be corrected statistically to a constant intake of food, nor can it be actually reduced by an equating of individual food intakes. Hence, the experimental errors of average measurements obtained from animals fed in groups are needlessly large.

The advantage of individual feeding is the possibility it presents of observing and controlling the behavior of each unit in a nutrition experiment. While there are advantages in merely observing this behavior, the greater the extent to which it is controlled the more nearly will the experiment yield results that are precise and susceptible of clear-cut interpretation.

The control of the consumption of food by experimental animals is of great importance in the production of a good nutrition experiment, unless, of course, the experiment is one designed to test an effect on appetite. Among animals on the same ration, the equalization of food intakes will reduce the experimental error of the average results for the group. Between two groups of animals subsisting on rations whose nutritive effects are being measured, an equalization of food consumption will establish a definite

relation between the one difference in composition of the rations and any statistically significant difference observed in animal functioning. Without such an equalization of food consumption the difference observed in animal functioning cannot be definitely related to the one difference in composition of the rations, because a difference in the amount of food eaten might alone affect animal function.

Since *ad libitum* feeding of experimental animals is so extensively practiced in nutrition laboratories, the bearing of this practice upon the interpretation of experimental results may well be considered here. Let us take as the simplest case, two experimental animals, one being fed one diet, which we will call the control diet, and the other being fed a second diet differing from the first in one particular only, which we will call the test diet. If these two animals are fed *ad libitum*, a difference in food intake during a feeding period of several weeks will always occur. We will assume that the animal on the test diet consumes the greater amount of food, and that it gains faster, or recuperates faster from a nutritional anemia, or deposits mineral in its skeletal tissue faster, or exhibits a more favorable functioning in some other respect, depending upon the purpose of the experiment. This more favorable functioning on the test diet could be referred directly to the difference in composition of the two diets were it not for another difference in experimental conditions, namely, the difference in the consumption of food. Of the possible causes for this difference in the consumption of food, we may consider the following:

1. It is the result of purely fortuitous causes, entirely unrelated to the different compositions of the diets: an expression of the "individuality" of the animals.
2. It is the result of a difference in the composition of the diets, either
   a. Unrelated to their inherent nutritive values, but in some way causing a differential stimulation of the appetite, or
   b. Related directly to their inherent nutritive values. In this case the test ration has been consumed in the greater amount because it is better balanced and better able to promote animal functioning, although in this case, as in *a*, the physiological mechanisms involved are not understood.

In each of these cases it would seem advisable to remove the effect of the difference in the consumption of food to permit the establishment of a direct relation between the difference in composition of the diets and the observed difference in animal functioning. In the first case, the difference in food intake is of the nature of an experimental error, being the result of uncontrolled factors. Its elimination could work only to the advantage of

the experiment. In the second case, under *a*, the difference in food consumption has been induced by dietary factors not concerned in the promotion of animal functioning. The observed difference in animal functioning may have been produced simply by the greater consumption of the test diet, or if the test diet is the better balanced, it is an exaggerated expression of this better balance. In either case an equating of the food intakes of the animals would have been advantageous in removing a confusing influence, either leading the unwary investigator to a false interpretation, or exaggerating the effect it is desired to measure.

In the last case, the better nutritive balance of the test ration has really produced two effects, namely, it has induced a greater consumption of food, and also, apart from the first, it has induced a more favorable physiological response. If the purpose of the experiment were to measure the difference in physiological response induced by these two diets, it would seem that this purpose would be better served by removing the former effect. The difference in physiological response would not be obscured by this control procedure. On the other hand, if it were desired to measure all nutritive differences of the two rations, the wiser procedure would seem to be to measure each separately. For the most effective progress, scientific problems should be factored into their ultimate terms.

In evaluating the importance of the control of the food consumption by experimental animals, which means limiting the intake of food of some of the animals at least below the amounts that they would voluntarily consume, it should be realized that most experiments in nutrition are concerned, not with obtaining and measuring the maximum nutritive effects of rations, but with measuring their comparative effects. While maximum effects can be obtained only under unrestricted conditions, comparative effects can be measured accurately only under the restricted conditions of a well-controlled experiment.

The interpretation of experimental data, as well as the control of experimental conditions, is not a simple problem nor one in the solution of which universal agreement can be expected. Surely the first step should be to enumerate all the possible interpretations. It will often be found that more than one interpretation is possible, but, if the experiment has been carefully planned and carried through, it should be found, either on the basis of the data obtained or on the basis of other information, that of all the possible interpretations one is considerably more probable than any of the others. The formulation of this outstanding interpretation into a definite conclusion doing full justice to the data without over-emphasizing their importance or significance is preëminently a judicial task.

The formulation of a positive conclusion from an experiment in which, for example, an effect of a given nutritive factor on animal functioning has been established, seems to be in a different category from the formulation of a negative conclusion from an experiment in which no such effect has been observed. In the former case, if the effect has been secured by the use of good technical methods and if it has been established on the basis of evidence that is statistically sound and adequate, it may be stated with considerable assurance that a causal relation has been established. For example, it may be stated definitely, if the above conditons are satisfied, that copper will stimulate hemoglobin production, or that carotin will induce recovery from the effects of vitamin A deficiency, or that certain nitrogenous extractives of meat will induce kidney damage. From the results of more exacting experiments it may be justifiable to conclude that the irradiation of ergosterol induces the production of vitamin D, or that the vitamin B complex consists of at least three constituents. These are positive conclusions which may be stated as such because any possible alternative explanations of the experimental data have been disposed of in a series of auxilliary experiments.

But what conclusion is justified when no effects are observed as the result of the imposition of a certain experimental condition? The choice lies between two conclusions: either the non-committal statement that no evidence has been obtained that the given condition is capable of producing an effect, or the outright statement that the given condition is incapable of producing an effect. Let us illustrate the situation. An investigation has been undertaken to find out whether the creatine content of muscle is affected by the amount of protein consumed. The significance of the question lies in the fact that arginine, one of the amino acids found in protein, is a probable precursor of the muscle extractive creatine. Two groups of rats are fed two different diets, one high in protein and one low in protein, and after 2 or 3 weeks all rats are sacrificed and selected muscles analyzed for creatine. No statistically significant difference is found between the creatine contents of the muscles of the two groups of rats. What conclusion should be drawn? Should it be said simply that no evidence has been obtained in favor of the view that the creatine content of muscles may be increased by high protein feeding, or should it be said that high protein feeding is incapable of increasing the creatine content of muscle? The latter statement can be shown to be untenable. In the first place it fails to consider that the experimental error, represented by the variations in creatine content among rats on the same diet, may have obscured a small effect of the high protein diet. Its greatest weakness, however, is in assuming that

under other conditions also no effect of protein feeding will ever be observed. It implies with no justification that negative results would also be obtained with rats of different age, with feeding periods of different duration, with different kinds of dietary protein, etc. A fair judgment would be, therefore, that if the conclusion from this negative experiment is thrown into a positive form, i.e., the assertion that high protein feeding is incapable of increasing the creatine content of muscle, the modifying clause "under the conditions of this experiment" should be appended.

Negative experimental evidence thus may be the result of any one of three possible circumstances:

1. The condition imposed has no causal relation with the thing measured, so that variation in the condition cannot exert any effect on the measurements taken.

2. The condition imposed has a small effect upon the measurements taken, but this effect is entirely obscured by the experimental error prevailing, either due to technical methods that are not sufficiently accurate or to an amount of data that is not statistically adequate.

3. The condition imposed has a considerable effect on the measurements taken, but the selection of other experimental conditions was unfortunate for the demonstration of a causal relation, because inadvertently some condition has been imposed under which the relation does not hold.

To establish the first interpretation the other two must be shown to be inapplicable. The removal of the second interpretation would seem to be purely a matter of technic, but actually it is impossible to say at any time that small or even infinitesimal effects have not been obscured by the variability in data secured under like conditions, or that small conversions of one substance into another in metabolism have not been missed. If such small effects are of no significance to the problem, then the second interpretation will not complicate matters.

In some problems, however, small effects are rather to be expected, and the failure to detect them gives no assurance that they do not exist. Until about 20 years ago chemical examination of the blood of animals made during the course of protein digestion failed to reveal any increase in the amino acid content, although it was known that amino acids were being liberated in the intestine. This purely negative evidence was unfortunately given a positive significance by concluding that amino acids do not gain access to the blood from the intestine. In explaining this imagined situation it was assumed that the amino acids resulting from protein digestion were resynthesized into protein in the intestinal walls during absorption,

and that these proteins were then taken up by the blood for the nourishment of the cells. Although no direct evidence was ever obtained in support of this theory, it gained wide currency because of the scientific prestige of its advocates, and it was not abandoned even when the only excuse for its inception was removed by the definite proof, by the use of better chemical methods, that amino acids are in fact absorbed as such from the intestinal tract.

Again, about sixteen years ago, two water-soluble vitamins were recognized, one the vitamin that prevents polyneuritis in animals and beri-beri in man, the other a growth-promoting vitamin. But at that time some rather perfunctory studies were made of the chemical properties of these vitamins and of their distribution in foods. The experimental methods employed were inadequate to afford a sharp distinction between the two vitamins in these respects and upon this purely negative evidence the belief was founded that the two vitamins were identical. For more than ten years this conclusion was accepted with very little question. But as the effectiveness of experimental methods increased data contradictory of this conclusion accumulated and more and more doubt was thrown upon this assumed identity, until but a few years ago it was definitely shown to be in error, and today the vitamins are again considered to be distinct. However, for thirteen years or more a large amount of experimental work was being carried out on a working hypothesis that had been insecurely based upon negative evidence. Today much of this work is unintelligible and represents almost a dead loss. The unfortunate part of this incident is that the lesson it should teach has not been perceived as generally as its importance justifies. Today many investigations are being conducted on the properties and distributions of these two components of the so-called "B complex" on the tacit assumption that no other components exist. If other components are later definitely characterized a majority of these studies also will be uninterpretable.

The third possible interpretation of negative experimental data is the greatest obstacle to the formulation of definite conclusions, because important biological facts may be obscured for reasons that may not be divulged by methodical experimentation. Investigations in the metabolism of creatine and creatinine are replete with illustrations of biochemical transformations that seem to occur only under severely circumscribed conditions the nature of which is not clear at the present time. In some cases, the nature of the obscuring conditions has been revealed. Only recently a group of investigators failed to obtain evidence that carotin could replace vitamin A

in nutrition and on this negative evidence they concluded that carotin was incapable of being converted into vitamin A. They explained the positive results obtained in other laboratories by showing that the carotin used there was not of the highest purity and by assuming that in all probability either it or the natural oils in which it was dissolved contained traces of vitamin A. More recently, however, they have reported that the error was in their own technic. In the first experiment they dissolved their carotin before administration to their rats in ethyl oleate, which they have since found undergoes a spontaneous oxidation in the course of which any carotin or vitamin A dissolved in the oil is destroyed. When highly purified carotin is given to the experimental rats dissolved in ethyl laurate, daily doses as small as .005 gram induced an appreciable gain in weight.

As a final illustration of the existence of conditions that obscure biological relations and prevent the securing of positive experimental results, the effect of sunlight on calcium deposition may be mentioned. In studies of calcium metabolism, no relation is demonstrable within wide limits between the calcium-to-phosphorus ratio in the food and the extent of calcification in the bones if the experimental animals have access to sunlight. In other words, negative results may be obtained. That these negative results do not mean that no relation exists would be evident when the experiments are repeated under conditions such that the animals have no access to direct sunlight.

This discussion of the interpretation of negative evidence does not imply that such evidence is valueless. In many cases it may be significant, but in all cases its interpretation should call for the exercise of extreme caution, particularly if other experiments have yielded good evidence possessing a contrary significance. The law of organic evolution cannot be overturned by the failure of the known facts and the theories of genetics to explain it, since evolutional transformations may depend on causes that have eluded even the precise and methodical experimentation of geneticists. Nor can it be said even that the Lamarckian theory of the inheritance of acquired characters can ever be disproved by showing that, in specific instances, acquired characters are not inherited.

In the best controlled experiments, unaccountable variation in measurements obtained under as nearly as possible the same conditions will always occur. This unaccountable variation, representing the experimental error, will always tend to discount to some extent the significance of differences in measurements obtained under different conditions and thus to

obscure the very effects it is desired to observe. In drawing any conclusion as to the existence or the magnitude of these effects, some fair allowance must be made for the influence of the unaccountable variation in measurements, representing uncontrolled or imperfectly controlled conditions. This is the function of statistical or biometrical methods, and their value in the interpretation of the data of biological experiments is difficult to over-emphasize.

In recent years an active development of biometrical methods has occurred and an application of them has been made to other problems than the mere calculation of probable errors, coefficients of correlation, etc. They may now be used to unravel, sometimes in a fairly satisfactory manner, the tangled and otherwise unintelligible data of imperfectly controlled experiments. This is all very fine, in that it permits the extraction from an experiment of the last particle of its information; but a real danger lies in the situation in so far as the existence of such methods of dealing with poorly controlled experiments may constitute an enticing invitation to slovenly experimental work. Suffice it to say that any experimental method that avoids a source of error is far to be preferred to another that trusts to probabilities for its elimination.

It should not be inferred, however, that the use of statistical methods in interpreting the results of a given experiment is a confession that the experiment was poorly controlled, though this inference is not infrequently made. Statistical methods have been put to greater use in the physical sciences where the control of experimental conditions can be much more complete, than in the biological sciences. In fact, the latest investigations of physicists concerning the fundamental nature of the universe indicate that the laws of probability, upon which statistical methods are based, are of more fundamental significance even than the law of causation. When the behavior of individual atoms or electrons is observed, it is found impossible to predict with certainty their positions at any given time. Determinism does not exist in events in which atoms and electrons are singly involved. But when atoms and electrons move in crowds their behavior is calculable by the laws of probability. In the same way, the throw of a single penny is quite unpredictable, but if we throw a million tons of pennies, we can predict that 500,000 tons of pennies will show heads and 500,000 tons will show tails. This illustration is borrowed from Sir James Jeans and concerning it he says:

"We may be tempted to instance it as evidence of the uniformity of na-

ture, and to infer the action of an underlying law of causation: in actual fact it is an instance only of the operation of the purely mathematical laws of chance."

Yet the number of pennies in a million tons "is nothing in comparison with the number of atoms in even the smallest piece of matter with which the earlier physicists could experiment. It is easy to see how the illusion of determinacy—if it is an illusion—crept into science."

H. H. M.

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